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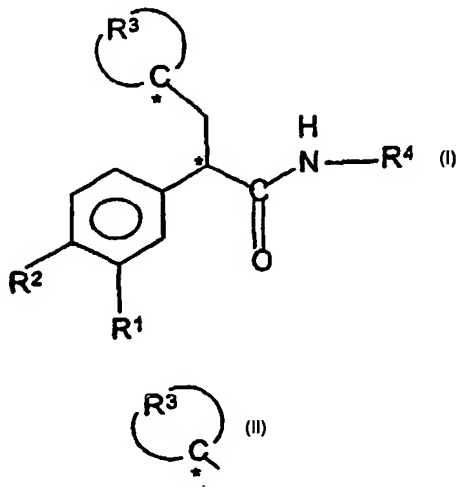
(54) Title: SUBSTITUTED PHENYLACETAMIDES AND THEIR USE AS GLUCOKINASE ACTIVATORS



(57) Abstract: Compounds of the formula (I); wherein formula (II) represents a substituted group, an oxa-cycloalkyl group or a thia-cycloalkyl group, are glucokinase activators useful in the treatment of type II diabetes.

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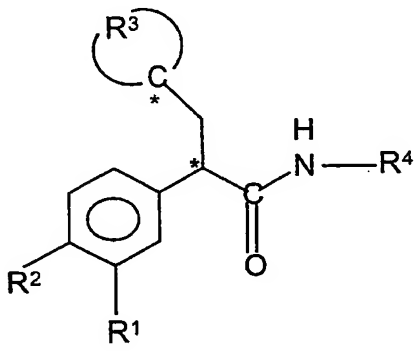
SUBSTITUTED PHENYLACETAMIDES AND THEIR USE AS GLUCOKINASE ACTIVATORS

[0001] Glucokinase (GK) is one of four hexokinases that are found in mammals [Colowick, S.P., in *The Enzymes*, Vol. 9 (P. Boyer, ed.) Academic Press, New York, NY, pages 1-48, 1973]. The hexokinases catalyze the first step in the metabolism of glucose, i.e., the conversion of glucose to glucose-6-phosphate. Glucokinase has a limited cellular distribution, being found principally in pancreatic β-cells and liver parenchymal cells. In addition, GK is a rate-controlling enzyme for glucose metabolism in these two cell types that are known to play critical roles in whole-body glucose homeostasis [Chipkin, S.R., Kelly, K.L., and Ruderman, N.B. in *Joslin's Diabetes* (C.R. Khan and G.C. Wier, eds.), Lea and Febiger, Philadelphia, PA, pages 97-115, 1994]. The concentration of glucose at which GK demonstrates half-maximal activity is approximately 8 mM. The other three hexokinases are saturated with glucose at much lower concentrations (<1 mM). Therefore, the flux of glucose through the GK pathway rises as the concentration of glucose in the blood increases from fasting (5 mM) to postprandial (≈10-15 mM) levels following a carbohydrate-containing meal [Printz, R.G., Magnuson, M.A., and Granner, D.K. in *Ann. Rev. Nutrition* Vol. 13 (R.E. Olson, D.M. Bier, and D.B. McCormick, eds.), Annual Review, Inc., Palo Alto, CA, pages 463-496, 1993]. These findings contributed over a decade ago to the hypothesis that GK functions as a glucose sensor in β-cells and hepatocytes (Meglasson, M.D. and Matschinsky, F.M. *Amer. J. Physiol.* **246**, E1-E13, 1984). In recent years, studies in transgenic animals have confirmed that GK does indeed play a critical role in whole-body glucose homeostasis. Animals that do not express GK die within days of birth with severe diabetes while animals overexpressing GK have improved glucose tolerance (Grupe, A., Hultgren, B., Ryan, A. et al., *Cell* **83**, 69-78, 1995; Ferrie, T., Riu, E., Bosch, F. et al., *FASEB J.*, **10**, 1213-1218, 1996). An increase in glucose exposure is coupled through GK

in β-cells to increased insulin secretion and in hepatocytes to increased glycogen deposition and perhaps decreased glucose production.

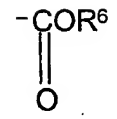
[0002] The finding that type II maturity-onset diabetes of the young (MODY-2) is caused by loss of function mutations in the GK gene suggests that GK also functions as a glucose sensor in humans (Liang, Y., Kesavan, P., Wang, L. et al., *Biochem. J.* **309**, 167-173, 1995). Additional evidence supporting an important role for GK in the regulation of glucose metabolism in humans was provided by the identification of patients that express a mutant form of GK with increased enzymatic activity. These patients exhibit a fasting hypoglycemia associated with an inappropriately elevated level of plasma insulin (Glaser, B., Kesavan, P., Heyman, M. et al., *New England J. Med.* **338**, 226-230, 1998). While mutations of the GK gene are not found in the majority of patients with type II diabetes, compounds that activate GK, and thereby increase the sensitivity of the GK sensor system, will still be useful in the treatment of the hyperglycemia characteristic of all type II diabetes. Glucokinase activators will increase the flux of glucose metabolism in β-cells and hepatocytes, which will be coupled to increased insulin secretion. Such agents would be useful for treating type II diabetes.

[0003] This invention provides a compound, comprising an amide of the formula:



I

wherein R^1 and R^2 are independently hydrogen, halo, amino, hydroxyamino, cyano, nitro, lower alkyl, $-OR^5$,



perfluoro-lower alkyl, lower alkyl thio, perfluoro-lower alkyl thio, lower alkyl sulfonyl, perfluoro-lower alkyl sulfonyl, lower alkyl sulfinyl, or sulfonamido;

R^3 is an unbranched alkyl chain of 4-5 carbon atoms or an unbranched heteroalkyl chain of 3-4 carbon atoms plus one oxygen or sulfur atom wherein the chain, in combination with the carbon atom it is bonded to, forms a five- or six-membered ring, and

when the chain contains no heteroatoms,

one carbon member of the chain is substituted with one moiety selected from the group consisting of hydroxy, oxo, hydroxyimino, methoxyimino, halo, methoxy, and acetoxy or

one carbon member of the chain is disubstituted with one hydroxy and one lower alkyl or is disubstituted with halogen

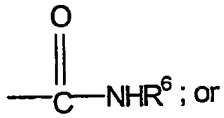
when the chain contains an O heteroatom,

the chain is unsubstituted, and

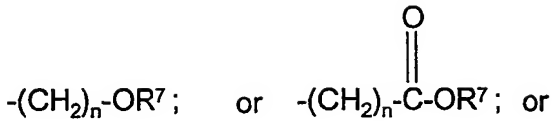
when the chain contains an S heteroatom,

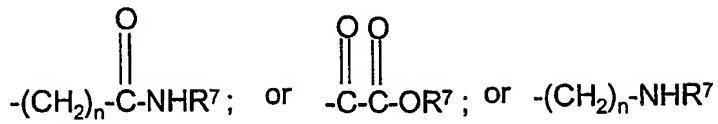
the chain is unsubstituted or the S heteroatom member of the chain is substituted by an oxo group;

R^4 is



an unsubstituted or mono-substituted five- or six-membered heteroaromatic ring connected by a ring carbon atom to the amine group shown, which five- or six-membered heteroaromatic ring contains from 1 to 3 heteroatoms selected from sulfur, oxygen or nitrogen, with one heteroatom being nitrogen which is adjacent to the connecting ring carbon atom; said mono-substituted heteroaromatic ring being mono-substituted at a position on a ring carbon atom other than adjacent to said connecting carbon atom with a substituent selected from the group consisting of lower alkyl, halo, nitro, cyano, perfluoro-lower alkyl, amidooxime, or





n is 0, 1, 2, 3 or 4;

R^5 is hydrogen, lower alkyl, or perfluoro-lower alkyl; R^6 is lower alkyl; and R^7 is hydrogen or lower alkyl;

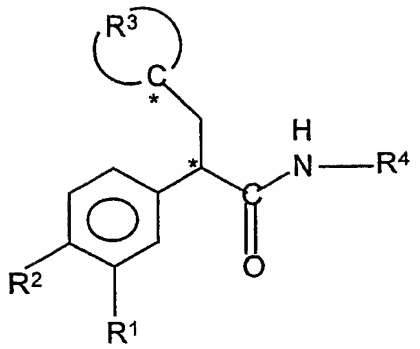
* denotes an asymmetric carbon atom;

or a pharmaceutically acceptable salt thereof.

[0004] The compounds of formula I have been found to activate glucokinase *in vitro*. Glucokinase activators are useful for increasing insulin secretion in the treatment of type II diabetes.

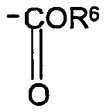
[0005] The present invention also relates to a pharmaceutical composition comprising a compound of formula I and a pharmaceutically acceptable carrier and/or adjuvant. Furthermore, the present invention relates to the use of such compounds as therapeutic active substances as well as to their use for the preparation of medicaments for the treatment or prophylaxis of type II diabetes. The present invention further relates to processes for the preparation of the compounds of formula I. In addition, the present invention relates to a method for the prophylactic or therapeutic treatment of type II diabetes, which method comprises administering a compound of formula I to a human being or an animal.

[0006] In more detail, this invention provides a compound, comprising an amide of the formula:



I

wherein R^1 and R^2 are independently hydrogen, halo, amino, hydroxyamino, cyano, nitro, lower alkyl, $-OR^5$,



perfluoro-lower alkyl, lower alkyl thio, perfluoro-lower alkyl thio, lower alkyl sulfonyl, perfluoro-lower alkyl sulfonyl, lower alkyl sulfinyl, or sulfonamido;

R^3 is an unbranched alkyl chain of 4-5 carbon atoms or an unbranched heteroalkyl chain of 3-4 carbon atoms plus one oxygen or sulfur atom, wherein the chain, in combination with the carbon atom it is bonded to, forms a five- or six-membered ring, and

when the chain contains no heteroatoms,

one carbon member of the chain is substituted with one moiety selected from the group consisting of hydroxy, oxo, hydroxyimino, methoxyimino, halo, methoxy, and acetoxy, or

one carbon member of the chain is disubstituted with one hydroxy and one lower alkyl or is disubstituted with halogen

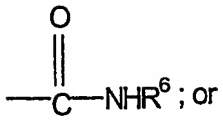
when the chain contains an O heteroatom,

the chain is unsubstituted, and

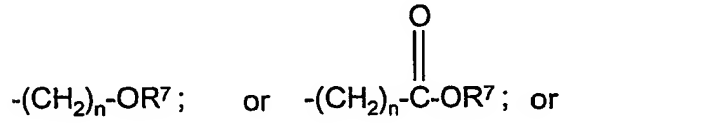
when the chain contains an S heteroatom,

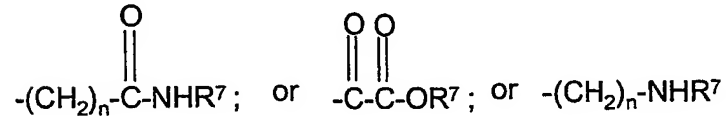
the chain is unsubstituted or the S heteroatom member of the chain is substituted by an oxo group;

R^4 is



an unsubstituted or mono-substituted five- or six-membered heteroaromatic ring connected by a ring carbon atom to the amine group shown, which five- or six-membered heteroaromatic ring contains from 1 to 3 heteroatoms selected from sulfur, oxygen or nitrogen, with one heteroatom being nitrogen which is adjacent to the connecting ring carbon atom; said mono-substituted heteroaromatic ring being mono-substituted at a position on a ring carbon atom other than adjacent to said connecting carbon atom with a substituent selected from the group consisting of lower alkyl, halo, nitro, cyano, perfluoro-lower alkyl, amidooxime, or





n is 0, 1, 2, 3 or 4;

R^5 is hydrogen, lower alkyl, or perfluoro-lower alkyl; R^6 is lower alkyl; and R^7 is hydrogen or lower alkyl;

* denotes a carbon atom that is asymmetric in all or most of the compounds of formula I;

or a pharmaceutically acceptable salt thereof.

[0007] In the compound of formula I, the "*" illustrates carbon atoms that are asymmetric in most or all of the species of formula I. The compound of formula I may be present either as a racemate or in isolated "R" or "S" configurations at the asymmetric carbons shown. The "R" enantiomers are preferred.

[0008] As used throughout this application, the term "lower alkyl" includes both straight chain and branched chain alkyl groups having from 1 to 7 carbon atoms, such as methyl, ethyl, propyl, isopropyl, preferably methyl and ethyl. As used herein, the term "halogen or halo" unless otherwise stated, designates all four halogens, i.e. fluorine, chlorine, bromine, and iodine. As used herein, "perfluoro-lower alkyl" means any lower alkyl group wherein all of the hydrogens of the lower alkyl group are substituted or replaced by fluoro. Among the preferred perfluoro-lower alkyl groups are trifluoromethyl, pentafluoroethyl, heptafluoropropyl, etc., with trifluoromethyl being especially preferred.

[0009] As used herein, the term "aryl" signifies aryl mononuclear aromatic hydrocarbon groups such as phenyl, tolyl, etc. which can be unsubstituted or substituted in one or more positions with halogen, nitro, lower alkyl, or lower alkoxy substituents and polynuclear aryl groups, such as naphthyl, anthryl, and phenanthryl, which can be unsubstituted or substituted with one or more of the aforementioned groups. Preferred aryl groups are the substituted and unsubstituted mononuclear aryl groups, particularly phenyl. As used herein, the term "lower alkoxy" includes both straight chain and branched chain alkoxy groups having from 1 to 7 carbon atoms, such as methoxy, ethoxy, propoxy, isopropoxy, preferably methoxy and ethoxy. The term "arylalkyl" denotes an alkyl group, preferably lower alkyl, in which one of the hydrogen atoms can be replaced by an aryl group. Examples of arylalkyl groups are benzyl, 2-phenylethyl, 3-phenylpropyl, 4-chlorobenzyl, 4-methoxybenzyl and the like.

[00010] As used herein, the term "lower alkanoic acid" denotes lower alkanoic acids containing from 2 to 7 carbon atoms such as propionic acid, acetic acid and the like. The term "lower alkanoyl" denotes monovalent alkanoyl groups having from 2 to 7 carbon atoms such as propionoyl, acetyl and the like. The term "aroic acids" denotes aryl alkanoic acids where aryl is as defined above and alkanoic contains from 1 to 6 carbon atoms. The term "aroyl" denotes aroic acids wherein aryl is as defined hereinbefore, with the hydrogen group of the COOH moiety removed. Among the preferred aroyl groups is benzoyl.

[0011] During the course of synthetic reactions, the various functional groups such as the free carboxylic acid or hydroxy groups may be protected *via* conventional hydrolyzable ester or ether protecting groups. As used herein, the term "hydrolyzable ester or ether protecting groups" designates any ester or ether conventionally used for protecting carboxylic acids or alcohols which can be hydrolyzed to yield the respective carboxy or hydroxy group. Exemplary ester groups useful for those purposes are those in which the acyl moieties are derived from a lower alkanoic, aryl lower alkanoic, or lower alkane dicarboxylic acid. Among the activated acids which can be utilized to form such groups are acid anhydrides, acid halides, preferably acid chlorides or acid bromides derived from aryl or lower alkanoic acids. Example of anhydrides are anhydrides derived from monocarboxylic acids such as acetic anhydride, benzoic acid anhydride, and lower alkane dicarboxylic acid anhydrides, e.g. succinic anhydride as well as chloro formates e.g. trichloromethyl chloroformate and ethyl chloroformate being preferred. A suitable ether protecting group for alcohols are, for example, the tetrahydropyranyl ethers such as 4-methoxy-5,6-dihydroxy-2H-pyranyl ethers. Others are aroylmethylethers such as benzyl, benzhydryl or trityl ethers or α-lower alkoxy lower alkyl ethers, for example, methoxymethyl or allylic ethers or alkyl silylethers such as trimethylsilylether.

[0012] The term "amino protecting group" designates any conventional amino protecting group which can be cleaved to yield the free amino group. The preferred protecting groups are the conventional amino protecting groups utilized in peptide synthesis. Especially preferred are those amino protecting groups which are cleavable under mildly acidic conditions from about pH 2 to 3. Particularly preferred amino protecting groups include t-butyl carbamate (BOC), benzyl carbamate (CBZ), and 9-flurorenylmethyl carbamate (FMOC).

[0013] The heteroaromatic ring defined by R^4 can be an unsubstituted or mono-substituted five- or six-membered heteroaromatic ring having from 1 to 3 heteroatoms selected from the group consisting of oxygen, nitrogen, or sulfur and connected by a ring carbon to the amine of the amide group shown. The

heteroaromatic ring contains a first nitrogen heteroatom adjacent to the connecting ring carbon atom, and if present, the other heteroatoms can be sulfur, oxygen, or nitrogen. Such heteroaromatic rings include, for example, pyridazinyl, isoxazolyl, isothiazolyl, and pyrazolyl. Among the preferred heteroaromatic rings are pyridinyl, pyrazinyl, pyrimidinyl, thiazolyl, oxazolyl, and imidazolyl. These heteroaromatic rings which constitute R^4 are connected *via* a ring carbon atom to the amide group to form the amides of formula I. The ring carbon atom of the heteroaromatic ring which is connected *via* the amide linkage to form the compound of formula I cannot contain any substituent.

[0014] When R^4 is an unsubstituted or mono-substituted five-membered heteroaromatic ring, the preferred rings are those which contain a nitrogen heteroatom adjacent to the connecting ring carbon and a second heteroatom adjacent to the connecting ring carbon or adjacent to said first heteroatom. The preferred five-membered heteroaromatic rings contain 2 or 3 heteroatoms with thiazolyl, imidazolyl, oxazolyl, and thiadiazolyl being especially preferred. When the heteroaromatic ring is a six-membered heteroaromatic, the ring is connected by a ring carbon to the amine group shown, with one nitrogen heteroatom being adjacent to the connecting ring carbon atom. The preferred six-membered heteroaromatic rings include, for example, pyridinyl, pyrimidinyl, pyrazinyl, pyridazinyl, and triazinyl.

[0015] The term "pharmaceutically acceptable salts" as used herein include any salt with both inorganic or organic pharmaceutically acceptable acids such as hydrochloric acid, hydrobromic acid, nitric acid, sulfuric acid, phosphoric acid, citric acid, formic acid, maleic acid, acetic acid, succinic acid, tartaric acid, methanesulfonic acid, *para*-toluene sulfonic acid and the like. The term "pharmaceutically acceptable salts" also includes any pharmaceutically acceptable base salt such as amine salts, trialkyl amine salts and the like. Such salts can be formed quite readily by those skilled in the art using standard techniques.

[0016] The five- or six-membered ring formed by the combination of R^3 and the carbon atom R^3 is attached to, hereinafter referred to as -CH<R^3, is a polar group. The five- or six-membered ring may consist of all carbon atoms or contain one heteroatom selected from oxygen or sulfur. If the five- or six-membered ring contains a heteroatom, all of the carbon atoms in the ring will be saturated with hydrogen atoms. If the heteroatom is S, then the S atom is optionally substituted with an oxo group. If the five- or six-membered ring contains no heteroatoms, one carbon member of the chain is substituted with one moiety selected from the group consisting of hydroxy, oxo, hydroxyimino, methoxyimino, halo, methoxy, and acetoxy or one carbon member of the chain is disubstituted with one hydroxy and one lower alkyl or is disubstituted with halogen. Such five- or six-membered rings include tetrahydro-furans, tetrahydro-pyrans, tetrahydro-thiopyrans, 1-oxo-tetrahydro-1-thiopyrans, keto-cycloalkyls, hydroxy-cycloalkyls, methoxy-cycloalkyls, hydroxyimino-cycloalkyls, methoxyimino-cycloalkyls, halo-cycloalkyls, and dihalo-cycloalkyls.

[0017] In accordance with one embodiment of the invention, called compound I-A,

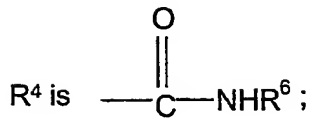
R^1 is hydrogen, halo, or perfluoro-lower alkyl;

R^2 is halo or lower alkyl sulfonyl;

R^3 is as defined above;

R^4 is an unsubstituted or mono-substituted five- or six-membered heteroaromatic ring connected by a ring carbon atom to the amine group shown, which five- or six-membered heteroaromatic ring contains from 1 or 2 heteroatoms selected from sulfur or nitrogen, with one heteroatom being nitrogen which is adjacent to the connecting ring carbon atom; said mono-substituted heteroaromatic ring being mono-substituted at a position on a ring carbon atom other than adjacent to said connecting carbon atom with a substituent selected from the group consisting of lower alkyl, lower alkoxy, halo, nitro, cyano, perfluoro-lower alkyl, amidooxime, or

$$-(CH_2)_n-OR^7, \quad \text{or} \quad -(CH_2)_n-\overset{\overset{O}{\|}}{C}-OR^7; \quad \text{or}$$



n is 0, 1, 2, 3 or 4; and

R^6 is lower alkyl; and R^7 is hydrogen or lower alkyl;

or a pharmaceutically acceptable salt thereof.

[0018] In other preferred embodiments of compound I-A, R^4 is -CO-NH-R^6 and R^3 is one of: a) a chain of 3 carbon atoms and one oxygen atom, b) a chain of 3 carbon atoms and one sulfur atom, c) a chain of 4 carbon atoms, d) a chain of 4 carbon atoms and one oxygen atom, e) a chain of 4 carbon atoms and one sulfur atom, or f) a chain of 5 carbon atoms. The groups -CH<R^3 may optionally be further substituted as defined above.

[0019] In still other preferred embodiments of compound I-A, R^4 is an unsubstituted thiazoyl and R^3 is one of: a) a chain of 3 carbon atoms and one oxygen atom, b) a chain of 3 carbon atoms and one sulfur atom, c) a chain of 4 carbon atoms, d) a chain of 4 carbon atoms and one oxygen atom, e) a chain of 4 carbon atoms and one sulfur atom, or f) a chain of 5 carbon atoms. The groups -CH<R^3 may optionally be further substituted as defined above.

[0020] In a further preferred embodiments of compound I-A, R^4 is an unsubstituted or mono-substituted pyrazinyl and R^3 is one of: a) a chain of 3 carbon atoms and one oxygen atom, b) a chain of 3 carbon atoms and one sulfur atom, c) a chain of 4 carbon atoms, d) a chain of 4 carbon atoms and one oxygen atom, e) a chain of 4 carbon atoms and one sulfur atom, or f) a chain of 5 carbon atoms. The groups -CH<R^3 may optionally be further substituted as defined above.

[0021] In yet further preferred embodiments of compound I-A, R^4 is a substituted pyridinyl and R^3 is one of: a) a chain of 3 carbon atoms and one oxygen atom, b) a chain of 3 carbon atoms and one sulfur atom, c) a chain of 4 carbon atoms, d) a chain of 4 carbon atoms and one oxygen atom, e) a chain of 4 carbon atoms and one sulfur atom, or f) a chain of 5 carbon atoms. The groups -CH<R^3 may optionally be further substituted as defined above.

[0022] In another preferable embodiment, the present invention relates to compounds of formula I, wherein R^1 and R^2 are independently hydrogen, halo such as chloro, perfluoro-lower alkyl such as trifluoromethyl, or lower alkyl sulfonyl such as methylsulfonyl; R^3 is an unbranched alkyl chain of 4-5 carbon atoms or an unbranched heteroalkyl chain of 3-4 carbon atoms plus one oxygen or sulfur atom, wherein the chain, in combination with the carbon atom it is bonded to, forms a five- or six-membered ring, and (1) when the chain contains no heteroatoms, one carbon member of the chain is substituted with one moiety selected from the group consisting of hydroxy, oxo, hydroxyimino, methoxyimino, halo such as fluoro, methoxy, and acetoxy; or one carbon member of the chain is disubstituted with one hydroxy and one lower alkyl such as methyl or is disubstituted with halogen such as fluoro; (2) when the chain contains an O heteroatom, the chain is unsubstituted; and (3) when the chain contains an S heteroatom, the chain is unsubstituted or the S heteroatom member of the chain is substituted by an oxo group; R^4 is –C(O)NHR^6 or an unsubstituted or mono-substituted five- or six-membered heteroaromatic ring connected by a ring carbon atom to the amine group shown, which five- or six-membered heteroaromatic ring contains from 1 or 2 heteroatoms selected from sulfur and nitrogen, with one heteroatom being nitrogen which is adjacent to the connecting ring carbon atom; said mono-substituted heteroaromatic ring being mono-substituted at a position on a ring carbon atom other than adjacent to said connecting carbon atom with a substituent selected from the group consisting of lower alkyl such as emthyl, halo such as chloro and bromo, cyano, amidooxime, or -$(CH_2)_n$-OR^7 or -$(CH_2)_n$-C(O)OR^7; n is 0 or 1; R^6 is lower alkyl such as methyl; R^7 is hydrogen or lower alkyl such as

methyl; * denotes a carbon atom that is asymmetric in all or most of the compounds of formula I; or a pharmaceutically acceptable salt thereof.

[0023] Preferably, R^1 and R^2 are each independently hydrogen, halo such as chloro, perfluoro-lower alkyl such as trifluoromethyl, or lower alkyl sulfonyl such as methylsulfonyl. More preferably, R^1 is hydrogen, halo such as chloro, or perfluoro-lower alkyl such as trifluoromethyl, and R^2 is halo such as chloro, or lower alkyl sulfonyl such as methylsulfonyl.

[0024] Preferred five- or six-membered rings formed by the combination of R^3 and the carbon atom R^3 is attached to are tetrahydrofuranyl such as tetrahydrofuran-2-yl and tetrahydrofuran-3-yl, tetrahydropyranyl such as tetrahydropyran-2-yl and tetrahydropyran-3-yl, tetrahydro-thiopyranyl such as terahydro-thiopyran-3(R)-yl and 1-oxo-hexahydro-1λ^4-thiopyran-3(R)-yl, and cycloalkyl such as cyclopentyl, 2-hydroxy-cyclopentyl, 3-hydroxy-cyclopentyl, 4-hydroxy-cyclopentyl, 2-oxo-cyclopentyl, 3-oxo-cyclopentyl, 4-oxo-cyclopentyl, 2-hydroxyimino-cyclopentyl, 3-hydroxyimino-cyclopentyl, 4-hydroxyimino-cyclopentyl, 2-methoxyimino-cyclopentyl, 3-methoxyimino-cyclopentyl, 4-methoxyimino-cyclopentyl, 2-fluorocyclopentyl, 3-methoxy-cyclopentyl, 3-acetoxy-cyclopentyl, 2,2-difluoro-cyclopentyl, 3,3-difluoro-cyclopentyl, 3-hydroxy-3-methyl-cyclopentyl.

[0025] In a preferable embodiment, R^4 is an unsubstituted or mono-substituted five- or six-membered heteroaromatic ring connected by a ring carbon atom to the amine group shown, which five- or six-membered heteroaromatic ring contains 1 or 2 heteroatoms selected from sulfur and nitrogen, with one heteroatom being nitrogen which is adjacent to the connecting ring carbon atom; said mono-substituted heteroaromatic ring being mono-substituted at a position on a ring carbon atom other than adjacent to said connecting carbon atom with a substituent selected from the group consisting of lower alkyl, such as methyl, halo, such as chloro or bromo, cyano, amidooxime, $-(CH_2)_n-OR^7$ or $-(CH_2)_n-C(O)OR^7$. In

another preferable embodiment, R^4 is a group $-C(O)NHR^6$, wherein R^6 is lower alkyl such as methyl.

[0026] Most preferred five- or six-membered heteroaromatic rings R^4 are thiazolyl, such as thazol-2-yl, pyrazinyl, such as pyrazin-2-yl, and pyridinyl, such as pyridin-2-yl. The said five- or six-membered heteroaromatic rings may optionally be mono-substituted at a position on a ring carbon atom other than adjacent to the connecting carbon atom with a substituent selected from the group consisting of lower alkyl such as methyl, halo such as chloro and bromo, cyano, amidooxime, $-(CH_2)_n-OR^7$ and $-(CH_2)_n-C(O)OR^7$, wherein n is 0 or 1 and R^7 is hydrogen, or lower alkyl such as methyl.

[0027] In another preferable embodiment, R^5 is hydrogen, lower alkyl such as methyl or perfluoro-lower alkyl such as trifluoromethyl.

[0028] In another preferable embodiment, R^6 is lower alkyl, such as methyl.

[0029] In another preferable embodiment, R^7 is hydrogen or lower alkyl such as methyl.

[0030] In another preferable embodiment, n is 0 or 1.

[0031] Preferable compounds in accordance with the present inevntion are selected from the group consisting of:

1-[2-(3,4-Dichloro-phenyl)-3-(tetrahydro-furan-2-yl)-propionyl]-3-methyl-urea,

1-[2-(3,4-Dichloro-phenyl)-3-(2-hydroxy-cyclopentyl)-propionyl]-3-methyl-urea,

1-[2-(3,4-Dichloro-phenyl)-3-(2-oxo-cyclopentyl)-propionyl]-3-methyl-urea,

1-[2-(3,4-Dichloro-phenyl)-3-(3-hydroxy-cyclopentyl)-propionyl]-3-methyl-urea,

1-[2-(3,4-Dichloro-phenyl)-3-(3-oxo-cyclopentyl)-propionyl]-3-methyl-urea,

1-[2-(3,4-Dichloro-phenyl)-3-(tetrahydro-pyran-2-yl)-propionyl]-3-methyl urea,

1-[2-(4-Methanesulfonyl-phenyl)-3-(tetrahydro-pyran-2-yl)-propionyl]-3-methyl-urea,

2-(3,4-Dichloro-phenyl)-3-(tetrahydro-furan-2-yl)-N-thiazol-2-yl-propionamide,

2-(4-Methanesulfonyl-phenyl)-3-(tetrahydro-furan-2(R)-yl)-N-thiazol-2-yl-propionamide,

2-(3,4-Dichloro-phenyl)-3-(2-hydroxy-cyclopentyl)-N-thiazol-2-yl-propionamide,

3-(2-Hydroxy-cyclopentyl)-2-(4-methanesulfonyl-phenyl)-N-thiazol-2-yl-propionamide,

2-(3,4-Dichloro-phenyl)-3-(2-oxo-cyclopentyl)-N-thiazol-2-yl-propionamide,

2-(4-Methanesulfonyl-phenyl)-3-(2-oxo-cyclopentyl)-N-thiazol-2-yl-propionamide,

2-(3,4-Dichloro-phenyl)-3-(3-hydroxy-cyclopentyl)-N-thiazol-2-yl-propionamide,

3-(3-Hydroxy-cyclopentyl)-2-(4-methanesulfonyl-phenyl)-N-thiazol-2-yl-propionamide,

2-(3,4-Dichloro-phenyl)-3-(3-methoxy-cyclopentyl)-N-thiazol-2-yl-propionamide,

3-[2-(3,4-dichloro-phenyl)-2-(thiazol-2-ylcarbamoyl)-ethyl]-cyclopentyl ester,

2-(3,4-Dichloro-phenyl)-3-(3-oxo-cyclopentyl)-N-thiazol-2-yl-propionamide,

2-(4-Methanesulfonyl-phenyl)-3-(3-oxo-cyclopentyl)-N-thiazol-2-yl-propionamide,

2-(3,4-Dichloro-phenyl)-3-(3-fluoro-cyclopentyl)-N-thiazol-2-yl-propionamide,

2-(3,4-Dichloro-phenyl)-3-(3-hydroxyimino-cyclopentyl)-N-thiazol-2-yl-propionamide,

2-(3,4-Dichloro-phenyl)-3-(3-methoxyimino-cyclopentyl)-N-thiazol-2-yl-propionamide,

2-(3,4-Dichloro-phenyl)-3-(2,2-difluoro-cyclopentyl)-N-thiazol-2-yl-propionamide,

2-(3,4-Dichloro-phenyl)-3-(3,3-difluoro-cyclopentyl)-N-thiazol-2-yl-propionamide,

2-(3,4-Dichloro-phenyl)-3-(tetrahydro-pyran-2-yl)-N-thiazol-2-yl-propionamide,

2(R)-(3,4-Dichloro-phenyl)-3-(tetrahydro-pyran-2-yl)-N-thiazol-2-yl-propionamide,

2-(4-Methanesulfonyl-phenyl)-3-(tetrahydro-pyran-2-yl)-N-thiazol-2-yl-propionamide,

2-(4-Methylsulfonyl-3-trifluoromethyl-phenyl)-3-(tetrahydro-pyran-2-yl)-N-thiazole-2-yl-propionamide,

6-[2-(4-Methylsulfonyl-3-trifluoromethyl-phenyl)-3-(tetrahydro-pyran-2-yl)-propionylamino]-nicotinic acid methyl ester,

6-[2-(4-Methylsulfonyl-3-trifluoromethyl-phenyl)-3-(tetrahydro-pyran-2-yl)-propionylamino]-nicotinic acid,

N-(5-Hydroxymethyl-pyridin-2-yl)-2-(4-methylsulfonyl-3-trifluoromethyl-phenyl)-3-(tetrahydro-pyran-2-yl)-propionamide,

2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-N-(5-chloro-pyridin-2-yl)-3-(4-oxo-cyclohexyl)-propionamide,

2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-N-(5-methyl-pyridin-2-yl)-3-(4-oxo-cyclohexyl)-propionamide,

2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-N-(5-chloro-pyridin-2-yl)-3-(4-hydroxyimino-cyclohexyl)-propionamide,

2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-3-(4-hydroxyimino-cyclohexyl)-N-(5-methyl-pyridin-2-yl)-propionamide,

2-(3-Chloro-4-methanesulfonyl-phenyl)-N-pyrazin-2-yl-3-(tetrahydro-furan-2(R)-yl)-propionamide,

2-(4-Methanesulfonyl-3-trifluoromethyl-phenyl)-N-pyrazin-2-yl-3-(tetrahydro-furan-2(R)-yl)-propionamide,

2-(3-Chloro-4-methanesulfonyl-phenyl)-N-pyrazin-2-yl-3-(tetrahydro-furan-3-yl)-propionamide,

2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-3-(2-oxo-cyclopentyl)-N-pyrazin-2-yl-propionamide,

2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-3-(2-hydroxy-cyclopentyl)-N-pyrazin-2-yl-propionamide,

2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-3-(3-hydroxy-cyclopentyl)-N-pyrazin-2-yl-propionamide,

2-(3-Chloro-4-methanesulfonyl-phenyl)-3-(3-oxo-cyclopentyl)-N-pyrazin-2-yl-propionamide,

2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-3-(3-oxo-cyclopentyl)-N-pyrazin-2-yl-propionamide,

2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-3-((S)-3-oxo-cyclopentyl)-N-pyrazin-2-yl-propionamide,

2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-3-((R)-3-oxo-cyclopentyl)-N-pyrazin-2-yl-propionamide,

N-(5-Bromo-pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(3-oxo-cyclopentyl)-propionamide,

2-(3-Chloro-4-methanesulfonyl-phenyl)-3-(2-hydroxyimino-cyclopentyl)-N-pyrazin-2-yl-propionamide,

2-(3-Chloro-4-methanesulfonyl-phenyl)-3-(2-methoxyimino-cyclopentyl)-N-pyrazin-2-yl-propionamide,

2-(3-Chloro-4-methanesulfonyl-phenyl)-3-(3-hydroxyimino-cyclopentyl)-N-pyrazin-2-yl-propionamide,

N-(5-Bromo-pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(3-hydroxyimino-cyclopentyl)-propionamide,

2-(3-Chloro-4-methanesulfonyl-phenyl)-3-(3-methoxyimino-cyclopentyl)-N-pyrazin-2-yl-propionamide,

N-(5-Bromo-pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(3-methoxyimino-cyclopentyl)-propionamide,

2-(3-Chloro-4-methanesulfonyl-phenyl)-3-(3-hydroxy-3-methyl-cyclopentyl)-N-pyrazin-2-yl-propionamide,

2-(4-Methanesulfonyl-3-trifluoromethyl-phenyl)-N-pyrazin-2-yl-3-(tetrahydro-pyran-2-yl)-propionamide,

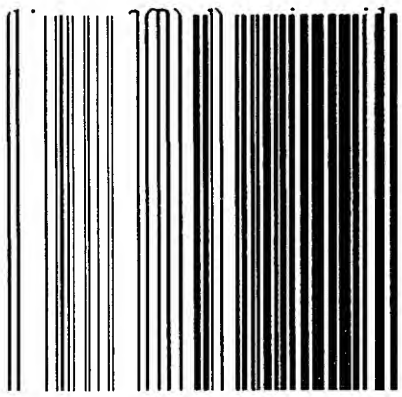
2-(3-Chloro-4-methanesulfonyl-phenyl)-N-pyrazin-2-yl-3-(tetrahydro-pyran-4-yl)-propionamide,

N-(5-Bromo-pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(tetrahydro-pyran-4-yl)-propionamide,

2-(3-Chloro-4-methanesulfonyl-phenyl)-N-(5-cyano-pyrazin-2-yl)-3-(tetrahydro-pyran-4-yl)-propionamide,

2-(3-Chloro-4-methanesulfonyl-phenyl)-N-[5-(N-hydroxycarbamimidoyl)-pyrazin-2-yl]-3-(tetrahydro-pyran-4-yl)-propionamide,

2-(3-Chloro-4-methanesulfonyl-phenyl)-N-pyrazin-2-yl-3-(tetrahydro-



2-(3-Chloro-4-methanesulfonyl-phenyl)-3-(4-oxo-cyclohexyl)-N-pyrazin-2-yl-propionamide,

N-(5-Bromo-pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(4-oxo-cyclohexyl)-propionamide,

N-(5-Bromo-pyrazin-2-yl)-2(R)-(3-chloro-4-methanesulfonyl-phenyl)-3-(4-oxo-cyclohexyl)-propionamide,

2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-N-(5-chloro-pyrazin-2-yl)-3-(4-oxo-cyclohexyl)-propionamide,

2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-N-(5-methyl-pyrazin-2-yl)-3-(4-oxo-cyclohexyl)-propionamide,

2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-N-(5-chloro-pyrazin-2-yl)-3-(4-hydroxy-cyclohexyl)-propionamide,

2-(3-Chloro-4-methanesulfonyl-phenyl)-3-(4-hydroxyimino-cyclohexyl)-N-pyrazin-2-yl-propionamide,

N-(5-Bromo-pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(4-hydroxyimino-cyclohexyl)-propionamide,

N-(5-Bromo-pyrazin-2-yl)-2(R)-(3-chloro-4-methanesulfonyl-phenyl)-3-(4-hydroxyimino-cyclohexyl)-propionamide,

2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-N-(5-chloro-pyrazin-2-yl)-3-(4-hydroxyimino-cyclohexyl)-propionamide,

2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-3-(4-hydroxyimino-cyclohexyl)-N-(5-methyl-pyrazin-2-yl)-propionamide,

2-(3-Chloro-4-methanesulfonyl-phenyl)-3-(4-methoxyimino-cyclohexyl)-N-pyrazin-2-yl-propionamide,

N-(5-Bromo-pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(4-methoxyimino-cyclohexyl)-propionamide,

and pharmaceutically acceptable salts thereof.

[0032] More preferable compounds in accordance with the present inevntion are selected from the group consisting of:

N-(5-Bromo-pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(tetrahydro-pyran-4-yl)-propionamide,

2-(3-Chloro-4-methanesulfonyl-phenyl)-N-(5-cyano-pyrazin-2-yl)-3-(tetrahydro-pyran-4-yl)-propionamide,

2-(3-Chloro-4-methanesulfonyl-phenyl)-N-[5-(N-hydroxycarbamimidoyl)-pyrazin-2-yl]-3-(tetrahydro-pyran-4-yl)-propionamide,

2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-3-(3-hydroxy-cyclopentyl)-N-pyrazin-2-yl-propionamide,

2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-3-(3-oxo-cyclopentyl)-N-pyrazin-2-yl-propionamide,

2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-3-((R)-3-oxo-cyclopentyl)-N-pyrazin-2-yl-propionamide,

N-(5-Bromo-pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(3-oxo-cyclopentyl)-propionamide,

N-(5-Bromo-pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(3-hydroxyimino-cyclopentyl)-propionamide,

2-(3-Chloro-4-methanesulfonyl-phenyl)-3-(3-methoxyimino-cyclopentyl)-N-pyrazin-2-yl-propionamide,

N-(5-Bromo-pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(3-methoxyimino-cyclopentyl)-propionamide,

N-(5-Bromo-pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(4-oxo-cyclohexyl)-propionamide,

N-(5-Bromo-pyrazin-2-yl)-2(R)-(3-chloro-4-methanesulfonyl-phenyl)-3-(4-oxo-cyclohexyl)-propionamide,

2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-N-(5-chloro-pyrazin-2-yl)-3-(4-oxo-cyclohexyl)-propionamide,

2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-N-(5-methyl-pyrazin-2-yl)-3-(4-oxo-cyclohexyl)-propionamide,

N-(5-Bromo-pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(4-hydroxyimino-cyclohexyl)-propionamide,

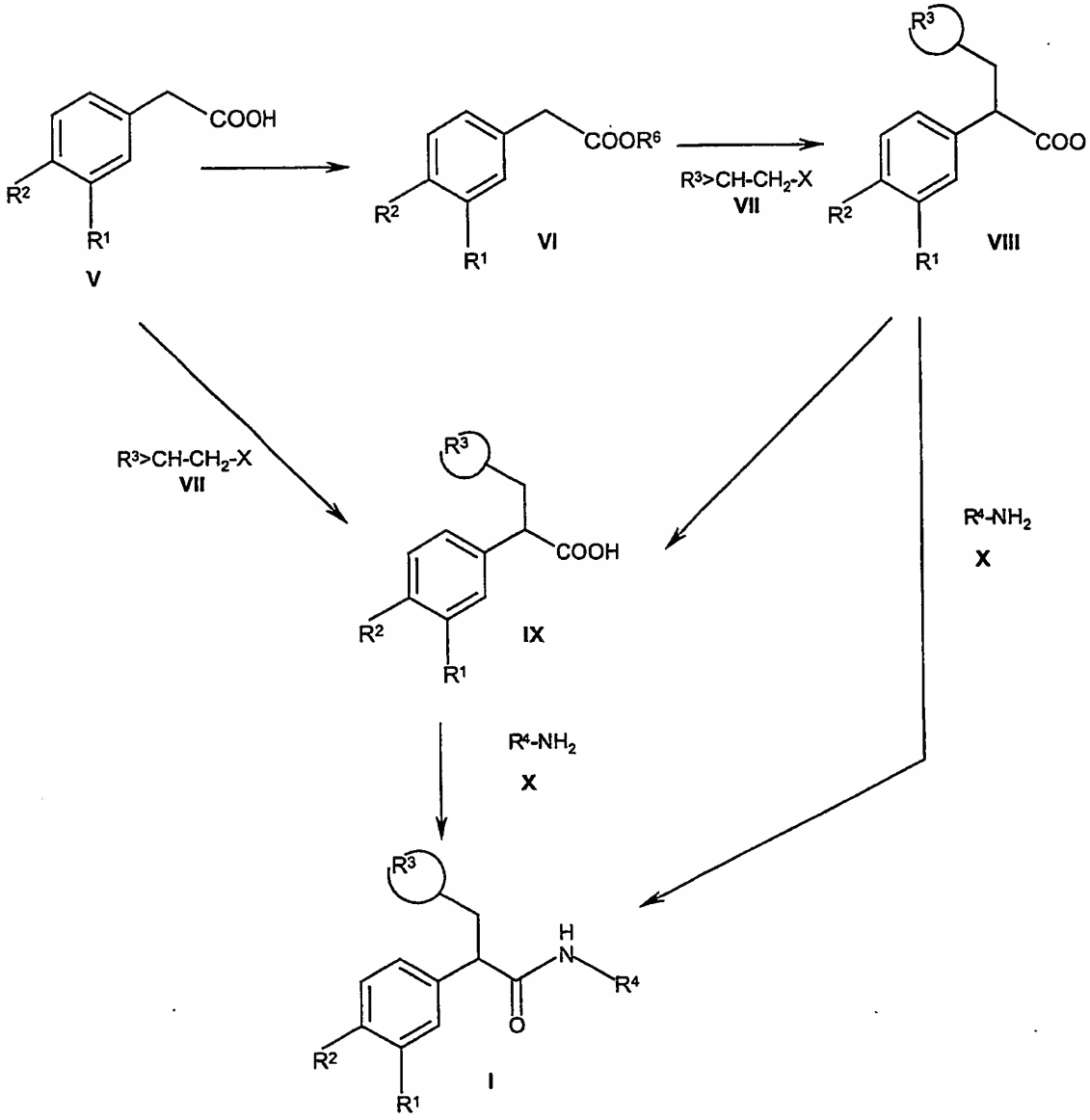
N-(5-Bromo-pyrazin-2-yl)-2(R)-(3-chloro-4-methanesulfonyl-phenyl)-3-(4-hydroxyimino-cyclohexyl)-propionamide,

2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-N-(5-chloro-pyrazin-2-yl)-3-(4-hydroxyimino-cyclohexyl)-propionamide,
and pharmaceutically acceptable salts thereof.

[0033] Most preferable compounds in accordance with the present invention are 2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-3-((R)-3-oxo-cyclopentyl)-N-pyrazin-2-yl-propionamide or 2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-3-(3-hydroxy-cyclopentyl)-N-pyrazin-2-yl-propionamide and optionally pharmaceutically acceptable salts thereof.

[0034] The compound of formula I can be prepared starting from the compound of formula V by the following Reaction Scheme:

Reaction Scheme



wherein R^1, R^2, R^3, R^4, and R^6 are as above.

[0035] The carboxylic acids or their lower alkyl esters of formula V and VI wherein one of R^1 and R^2 is hydrogen, nitro, mercapto, methylthio, trifluoromethylthio, methylsulfonyl, amino, fluoro, chloro, bromo, iodo, hydroxy, methoxy, trifluoromethoxy, methyl, trifluoromethyl, and carboxy, and the other is hydrogen are commercially available. In cases where only the carboxylic acids are available, and if necessary for further chemical modification to produce the desired substitutions at R^1 and R^2, the carboxylic acids can be converted to the

corresponding esters of lower alkyl alcohols using any conventional esterification methods.

[0036] All the reactions hereto forward are to be carried out on the lower alkyl esters of the carboxylic acids of formula VI or VIII or may be carried out on the carboxylic acids of formula V or IX themselves.

[0037] The compounds of formula V where one of R^1 and R^2 is amino can be converted to other substituents either before or after conversion to the compounds of formula I. In this respect, the amino groups can be diazotized to yield the corresponding diazonium compound, which *in situ* can be reacted with the desired lower alkyl thiol or perfluoro-lower alkyl thiol (see for example, Baleja, J.D. *Synth. Comm.* **1984**, *14*, 215; Giam, C. S.; Kikukawa, K., *J. Chem. Soc, Chem. Comm.* **1980**, 756; Kau, D.; Krushniski, J. H.; Robertson, D. W, *J. Labelled Compd Rad.* **1985**, *22*, 1045; Oade, S.; Shinhama, K.; Kim, Y. H., *Bull Chem Soc. Jpn.* **1980**, *53*, 2023; Baker, B. R.; et al, *J. Org. Chem.* **1952**, *17*, 164) to yield corresponding compounds of formula V where one of the substituents is lower alkyl thio or perfluoro-lower alkyl thio and the other is hydrogen. If desired, the lower alkyl thio or perfluoro-lower alkyl thio compounds can then be converted to the corresponding lower alkyl sulfonyl or perfluoro-lower alkyl sulfonyl substituted compounds of formula V by oxidation. Any conventional method of oxidizing alkyl thio substituents to sulfones can be utilized to effect this conversion. On the other hand, the lower alkyl thio compounds can also be converted to the corresponding lower alkyl sulfinyl compounds of formula V by oxidation. Any conventional method of oxidizing alkyl thio substituents to sulfoxides can be utilized to effect this conversion.

[0038] If it is desired to produce compounds of formula V where one of R^1 and R^2 is hydrogen and the other is sulfonamido, the mercapto substituent can be oxidized to a $-SO_3H$ group which then can be converted to $-SO_2Cl$, which, in turn, is then reacted with ammonia to form the sulfonamide substituent, $-SO_2-NH_2$.

[0039] If it is desired to produce compounds with lower alkyl or perfluoro-lower alkyl groups of compounds of formula V, the corresponding halo substituted compounds of formula V can be used as starting materials. Any conventional method of converting an aromatic halo group to the corresponding alkyl group (see for example, Katayama, T.; Umeno, M., *Chem. Lett.* **1991**, 2073; Reddy, G. S.; Tam., *Organometallics*, **1984**, *3*, 630; Novak, J.; Salemink, C. A., *Synthesis*, **1983**, *7*, 597; Eapen, K. C.; Dua, S. S.; Tamboroski, C., *J. Org. Chem.* **1984**, *49*, 478; Chen, Q, -Y.; Duan, J. –X. *J. Chem. Soc. Chem. Comm.* **1993**, 1389; Clark, J. H.; McClinton, M. A.; Jone, C. W.; Landon, P.; Bisohp, D.; Blade, R. J., *Tetrahedron Lett.* **1989**, 2133; Powell, R. L.; Heaton, C. A, US patent 5113013) can be utilized to effect this conversion.

[0040] For compounds of formula V wherein one or both of R^1 and R^2 is hydroxyamino, the corresponding nitro compounds can be used as starting material and can be converted to the corresponding compounds where R^1 and/or R^2 are hydroxyamino. Any conventional method of converting a nitro group to the corresponding aromatic hydroxyamino compound can be used to affect this conversion.

[0041] The carboxylic acids or esters of formula V or VI wherein both of R^1 and R^2 are chloro, fluoro, hydroxy, and methoxy are commercially available. The carboxylic acid of formula V wherein R^1 is trifluoromethyl and R^2 is fluoro, and the carboxylic acid of formula V wherein R^1 is nitro and R^2 is chloro are also commercially available. In cases, where only the carboxylic acids are available, they can be converted to the corresponding esters of lower alkyl alcohols using any conventional esterification method.

[0042] To produce the compound of formula V where both R^1 and R^2 are nitro, 3,4-dinitrotoluene can be used as starting material. This compound can be converted to the corresponding 3,4-dinitrophenyl acetic acid. Any conventional method of converting an aryl methyl group to the corresponding aryl acetic acid can be utilized to effect this conversion (see for example, Clark, R. D.; Muchowski, J. M.; Fisher, L. E.; Flippin, L. A.; Repke, D. B.; Souchet, M, *Synthesis*, **1991**, 871).

The compounds of formula V where both R^1 and R^2 substituents are amino can be obtained from the corresponding dinitro compound of formula V, described above. Any conventional method of reducing a nitro group to an amine can be utilized to effect this conversion.

[0043] The compound of formula V where both R^1 and R^2 are amine groups can be used to prepare the corresponding compound of formula V where both R^1 and R^2 are iodo or bromo via a diazotization reaction. Any conventional method of converting an amino group to an iodo or bromo group (see for example, Lucas, H. J.; Kennedy, E. R. *Org. Synth. Coll. Vol, II* **1943**, 351) can be utilized to effect this conversion.

[0044] If it is desired to produce compounds of formula V where both R^1 and R^2 are lower alkyl thio or perfluoro-lower alkyl thio groups, the compound of formula V where R^1 and R^2 are amino can be used as starting material. Any conventional method of converting an aryl amino group to an aryl lower alkyl thio or to a perfluoro-lower alkyl thio group can be utilized to effect this conversion. If it is desired to produce compounds of formula V where R^1 and R^2 are lower alkyl sulfonyl or lower perfluoro alkyl sulfonyl, the corresponding compounds of formula V where R^1 and R^2 are lower alkyl thio or perfluoro-lower alkyl thio can be used as starting material. Any conventional method of oxidizing alkyl thio substituents to sulfones can be utilized to effect this conversion. On the other hand, if it is desired to produce compounds of formula V where R^1 and R^2 are lower alkyl sulfinyl, the corresponding compounds of formula V where R^1 and R^2 are lower alkyl thio can be used as starting material. Any conventional method of oxidizing alkyl thio substituents to sulfoxides can be utilized to effect this conversion.

[0045] If it is desired to produce compounds of formula V where both R^1 and R^2 are substituted with lower alkyl or perfluoro-lower alkyl groups, the corresponding halo substituted compounds of formula V can be used as starting materials. Any conventional method of converting an aromatic halo group to the corresponding lower alkyl or perfluoro-lower alkyl group can be utilized to effect this conversion.

[0046] The carboxylic acids corresponding to the compounds of formula V where one of R^1 and R^2 is nitro and the other is halo are known from the literature (see for 4-chloro-3-nitrophenyl acetic acid: Tadayuki, S.; Hiroki, M.; Shinji, U.; Mitsuhiro, S. Japanese patent, JP 71-99504, *Chemical Abstracts* 80:59716; see for 4-nitro-3-chlorophenyl acetic acid: Zhu, J.; Beugelmans, R.; Bourdet, S.; Chastanet, J.; Rousssi, G. *J. Org. Chem.* **1995**, *60*, 6389; Beugelmans, R.; Bourdet, S.; Zhu, J. *Tetrahedron Lett.* **1995**, *36*, 1279). These carboxylic acids can be converted to the corresponding lower alkyl esters using any conventional esterification methods. Thus, if it is desired to produce the compound of formula V where one of R^1 and R^2 is nitro and the other is lower alkyl thio or perfluoro-lower alkyl thio, the corresponding compound where one of R^1 and R^2 is nitro and the other is chloro can be used as starting material. In this reaction, any conventional method of nucleophilic displacement of an aromatic chlorine group with a lower alkyl thiol or perfluoro-lower alkyl thio can be used (see for example, Singh, P.; Batra, M. S.; Singh, H, *J. Chem. Res.-S* **1985** (6), S204; Ono, M.; Nakamura, Y.; Sata, S.; Itoh, I, *Chem. Lett*, **1988**, 1393; Wohrle, D.; Eskes, M.; Shigehara, K.; Yamada, A, *Synthesis*, **1993**, 194; Sutter, M.; Kunz, W, US patent, US 5169951). Once the compounds of formula V where one of R^1 and R^2 is nitro and the other is lower alkyl thio or perfluoro-lower alkyl thio are available, they can be converted to the corresponding compounds of formula V where one of R^1 and R^2 is nitro and the other is lower alkyl sulfonyl or perfluoro-lower alkyl sulfonyl using conventional oxidation procedures. If it is desired to produce compounds of formula V where one of R^1 and R^2 is amino and the other is lower alkyl thio or perfluoro-lower alkyl thio, the corresponding compound where one of R^1 and R^2 is nitro and the other is lower alkyl thio or perfluoro-lower alkyl thio can be used as starting materials. Any conventional method of reducing an aromatic nitro group to an amine can be utilized to effect this conversion. If it is desired to produce compounds of formula V where one of R^1 and R^2 is lower alkyl thio and the other is perfluoro-lower alkyl thio, the corresponding compound where one of R^1 and R^2 is amino and the other is lower alkyl thio or perfluoro-lower alkyl thio can be used as starting materials. Any conventional method of diazotizing an aromatic amino group and reacting it *in situ* with the desired lower alkyl thiol can be utilized to

effect this conversion. If it is desired to produce compounds of formula V where one of R^1 and R^2 is lower alkyl sulfonyl and the other is perfluoro-lower alkyl sulfonyl, the corresponding compounds where one of R^1 and R^2 is lower alkyl thio and the other is perfluoro-lower alkyl thio can be used as starting materials. Any conventional method of oxidizing an aromatic thio group to the corresponding sulfone group can be utilized to effect this conversion.

[0047] If it is desired to produce compounds of formula V where one of R^1 and R^2 is halo and the other is lower alkyl thio or perfluoro-lower alkyl thio, the corresponding compounds where one of R^1 and R^2 is amino and the other is lower alkyl thio or perfluoro-lower alkyl thio can be used as starting materials. Any conventional method of diazotizing an aromatic amino group and conversion of it *in situ* to an aromatic halide can be utilized to effect this conversion. If it is desired to produce compounds of formula V where one of R^1 and R^2 is halo and the other is lower alkyl sulfonyl or perfluoro-lower alkyl sulfonyl, the corresponding compounds where one of R^1 and R^2 is halo and the other is lower alkyl thio or perfluoro-lower alkyl thio can be used as starting materials. Any conventional method of oxidizing an aromatic thio group to the corresponding sulfone can be utilized to effect this conversion.

[0048] If one wishes to prepare the compound formula V where one of R^1 and R^2 is nitro and the other is amino, the compound of formula V where one of R^1 and R^2 is nitro and the other is chloro can be used as a starting material. The chloro substituent on the phenyl ring can be converted to an iodo substituent (see for example, Bunnett, J. F.; Conner, R. M.; *Org. Synth. Coll Vol V*, **1973**, 478; Clark, J. H.; Jones, C. W. *J. Chem. Soc. Chem. Commun.* **1987**, 1409), which in turn can be reacted with an azide transferring agent to form the corresponding azide (see for example, Suzuki, H.; Miyoshi, K.; Shinoda, M. *Bull. Chem. Soc. Jpn*, **1980**, *53*, 1765). This azide can then be reduced in a conventional manner to form the amine substituent by reducing it with commonly used reducing agents for converting azides to amines (see for example, Soai, K.; Yokoyama, S.; Ookawa, A. *Synthesis*, **1987**, 48).

[0049] If it is desired to produce the compound of formula V where both R^1 and R^2 are cyano, this compound can be prepared as described hereinbefore from compounds where R^1 and R^2 are amino via diazotization to produce the diazonium salt followed by reaction with a cyano group transferring agent. If it is desired to produce compounds of formula V where one of R^1 and R^2 is cyano and the other is not cyano, the compound of formula V where one of R^1 and R^2 is nitro and the other is chloro can be used as a starting material. Using this starting material, the nitro is first reduced to the amino derivative. Any conventional method of reducing a nitro group to an amine can be utilized to effect this conversion. The amino group is then converted to the cyano group via diazotization to produce the diazonium salt followed by reaction with a cyano group transferring agent. The halo can then be converted to any other desired R^1 and R^2 substituent as described hereinbefore.

[0050] If it is desired to produce the compound of formula V wherein one of R^1 or R^2 is a -C(O)-OR^6, this compound can be formed from the corresponding compound where one of R^1 and R^2 is an amino group by converting the amino group to a diazonium salt, reacting the diazonium salt with a hydrohalic acid to form the corresponding halide, forming the Grignard reagent from the corresponding halide, and finally reacting the Grignard reagent with a carboxylate source to produce the corresponding acid which can then be esterified. On the other hand, if one wants to produce the compound of formula V where both R^1 and R^2 are -C(O)-OR^6, this compound can be produced as described above from the corresponding compound of formula V where both R^1 and R^2 are amino groups. In the same manner, the amino groups in the compound of formula V can be converted to the corresponding compound where either R^1 or R^2 or both of R^1 and R^2 is OR^5 by simply reacting the amino group with sodium nitrate in sulfuric acid to convert the amino group to a hydroxy group and thereafter etherifying, if desired, the hydroxy group.

[0051] If it is desired to produce compounds of formula V where R^1 is hydrogen and R^2 is lower alkyl sulfonyl, the known 4-mercaptophenylacetic acid may be used as a starting material. The compound of formula V where R^1 is hydrogen and R^2 is

mercapto may be alkylated by conventional methods (for example, with an alkyl halide) to the corresponding lower alkyl thio compounds of formula V. The lower alkyl thio compounds can then be converted to the corresponding lower alkyl sulfonyl compounds of formula V by oxidation. Any conventional method of oxidizing an alkyl thio substituent to the corresponding sulfone group can be utilized to effect this conversion.

[0052] On the other hand, if it is desired to produce the compounds of formula V where R^1 is trifluoromethyl and R^2 is lower alkyl sulfonyl, the known 4-fluoro-3-(trifluoromethyl)phenyl acetic acid can be used as a starting material. In this reaction, any conventional method of nucleophilic displacement of an aromatic fluorine group with a lower alkyl thiol can be utilized to effect this conversion (see for example, Boswell, G. E.; Licause, J. F. *J. Org. Chem.* **1995**, 6592; Sheikh, Y. M. et al. *J. Org. Chem.* **1982**, 4341; Brown, F. C. et al. *J. Org. Chem.* **1961**, 4707). Once the compounds of formula V where R^1 is trifluoromethyl and R^2 is lower alkyl thio are available, they can be converted to the corresponding compounds of formula V where R^1 is trifluoromethyl and R^2 is lower alkyl sulfonyl using conventional oxidation procedures.

[0053] If it is desired to produce compounds of formula V where R^1 is halo and R^2 is lower alkyl sulfonyl, the known 2-halothiophenols can also be used as starting material. In this reaction sequence, the mercapto group may be alkylated by conventional methods (for example, with a lower alkyl halide) to the corresponding 2-halo-1-lower alkyl thio benzenes. These compounds can then be converted to the corresponding 3-halo-4-(lower alkyl thio)-phenyl acetic acids. First, the 2-halo-1-lower alkyl thio benzenes are acylated with a (lower alkyl)oxalyl chloride (such as methyloxalyl chloride or ethyloxalyl chloride) via a Friedel-Crafts acylation to produce the alpha-keto carboxylic ester in the position *para* to the lower alkyl thio functional group. The alpha-keto carboxylic ester is next hydrolyzed by any conventional method to convert a alpha-keto carboxylic ester to a alpha-keto carboxylic acid. Wolff-Kishner reduction of the resulting alpha-keto carboxylic acid will produce the compounds of formula V where R^1 is halo and R^2 is lower alkyl thio (see for example, Levine, S. D. *J. Med. Chem.*

1972, 1029 for a similar reaction sequence). The lower alkyl thio compounds can then be converted to the corresponding lower alkyl sulfonyl compounds of formula V by oxidation. Any conventional method of oxidizing an alkyl thio substituent to the corresponding sulfone group can be utilized to effect this conversion.

[0054] For the alkylation reaction using the alkyl halide of formula VII, the carboxylic acids of formula V can be directly alkylated or first converted to the corresponding esters of lower alkyl alcohols of formula VI using any conventional esterification methods and then alkylated. In the alkylation step of the Reaction Scheme, the alkyl halide of formula VII is reacted with the dianion of formula V to produce the compound of formula IX or reacted with the anion of formula VI to produce the compound of formula VIII. The compounds of formula V and VI represent an organic acid and an organic acid derivative having an alpha carbon atom, and the compound of formula VII is an alkyl halide so that alkylation occurs at the alpha carbon atom of this carboxylic acid. This reaction is carried out by any conventional means of alkylation of the alpha carbon atom of a carboxylic acid or a lower alkyl ester of a carboxylic acid. Generally, in these alkylation reactions, an alkyl halide is reacted with the dianion of the acetic acid or the anion generated from an acetic acid ester. The anion can be generated by using a strong organic base such as lithium diisopropylamide or *n*-butyl lithium as well as other organic lithium bases. In carrying out this reaction, low boiling ether solvents are utilized such as tetrahydrofuran at low temperatures, from -80°C to about -10°C being preferred. However, any temperature from -80°C to room temperature can be used. If necessary, the alkylation reactions may proceed using a triflate alkylation subunit instead of the halo alkylation subunit of compound VII. These triflate alkylation reactions can be preformed according to procedures well-known in the art of synthetic organic chemistry.

[0055] If it is desired to produce the compound of formula I where R^4 is CONH-R^6 and R^6 is lower alkyl, the methyl ester of formula VIII is reacted with a lower alkyl urea to produce the compound of forumula I. This reaction is carried out by utilizing any conventional means of reacting a methyl ester with a lower alkyl urea to form the corresponding condensation product. The required lower alkyl ureas

are commercially available (for example, methylurea, ethylurea *n*-propylurea, *n*-butyulurea) or are known in the chemical literature.

[0056] Where it is desired to produce the compound of formula I where R^4 is an unsubstituted or mono-substituted five- or six-membered heteroaromatic ring, the compound of formula VIII can be converted to the compound of formula IX by any conventional procedure to convert a carboxylic acid ester to an acid. The compound of formula IX is then condensed with the compounds of formula X *via* conventional peptide coupling to produce the compounds of formula I. In carrying out this reaction, any conventional method of condensing a primary amine with a carboxylic acid can be utilized to effect this conversion. On the other hand, the compound of formula VIII can also be condensed with the compound of formula X *via* conventional procedures to produce the compound of formula I. In carrying out this reaction, any conventional method of condensing a primary amine with a carboxylic acid ester can be utilized to effect this conversion.

[0057] The amino heteroaromatic compounds of formula X are commercially available, or are known in the chemical literature, or can be prepared from those skilled in the art by using adaptions of standard synthetic transformations reported in the chemical literature. For example, the heteroaromatics of formula X, wherein one of the substitutions is $-(CH_2)_nCOOR^7$, where n = 1, 2, 3, or 4 and R^7 is hydrogen or lower alkyl can be prepared from the corresponding carboxylic acid $-(CH_2)_nCOOR^7$ (n = 0 and R^7 is hydrogen). Any conventional carbon homologation method can be utilized to convert a lower carboxylic acid to its higher homologs (see for example, Skeean, R. W.; Goel, O. P. *Synthesis*, **1990**, 628), which in turn can then can be converted to the corresponding lower alkyl esters using any conventional esterification methods. The heteroaromatics of formula X, wherein one of the substitutions is $-(CH_2)_nC(=O)NHR^7$, where n = 0, 1, 2, 3, or 4 and R^7 is hydrogen or lower alkyl can in turn be made by the above mentioned carboxylic acids. Any conventional means of converting carboxylic acids to the corresponding amides may be utilited to effect this conversion. In turn, the lower alkyl amides can be converted to the corresponding amines of formula X, wherein one of the substitutions is $-(CH_2)_nNHR^7$, by any conventional

amide reduction method. The heteroaromatics of formula X, wherein one of the claimed substitutions is $-(CH_2)_nOR^7$, where n = 1, 2, 3, or 4 can be prepared from the above said corresponding lower alkyl esters. The lower alkyl esters can be converted to the corresponding alcohols using any conventional ester reduction method.

[0058] Such amines and alcohols described above would have to be selectively protected before carrying out the condensation step. The amino group and alcohol group can be protected with any conventional acid removable group. The protecting groups are then removed from the amine and alcohol groups after the coupling step to produce the desired compounds of formula I.

[0059] The heteroaromatics of formula X, wherein one of the substituents is $-C(O)C(O)OR^7$ or $-C(O)-OR^7$ and R^7 is lower alkyl, can be prepared from the corresponding halogen. Any conventional acylation method to convert an aromatic or heteroaromatic halogen to its oxoacetic acid lower ester or ester derivative (see for example, Hayakawa, K.; Yasukouchi, T.; Kanematsu, K. *Tetrahedron Lett*, **1987**, *28*, 5895) can be utilized. On the other hand, if it is desired to produce compounds with lower alkyl or perfluoro-lower alkyl groups of compounds of formula X, the corresponding halo substituted compounds of formula X can be used as starting materials. Any conventional method of converting an aromatic halo group to the corresponding lower alkyl group or perfluoro-lower alkyl group can be utilized to effect this conversion.

[0060] If it is desired to produce the heteroaromatic of formula X wherein one of the substitutions is cyano or the compound of formula I wherein one of the substitutions on the five- or six-membered heteroaromatic ring is cyano, then the corresponding halogen (especially bromo) can be utilized as the starting material. Any conventional method of converting a halogen to a cyanide may be utilized to effect this conversion. On the other hand, if it is desired to produce the compound of formula I wherein one of the substitutions on the five- or six-membered heteroaromatic ring is amidooxime, it is best to form this functional group after the condensation step from the corresponding cyano group. Any conventional

method of amidooxime formation from a cyano can be utilized to effect this conversion.

[0061] The five- or six-membered ring formed by the combination of R^3 and the carbon atom R^3 is attached to, hereinafter referred to as -CH<R^3, is a polar group. The five- or six-membered ring may consist of all carbon atoms or contain one heteroatom selected from oxygen or sulfur.

[0062] If it is desired to produce the compound of formula VIII or IX in which -CH<R^3 is 2-tetrahydrofuran, the commercially available starting material, 2-bromomethyl-tetrahydrofuran, may be used as the alkyl halide for the alkylation step. Any conventional means of alkylation of the alpha carbon atom of a carboxylic acid or a lower alkyl ester of a carboxylic acid may be utilized to effect this conversion. Another chemical means to produce the compound of formula VIII or IX in which -CH<R^3 is 2-tetrahydrofuran is from the commercially available alcohol, (tetrahydro-furan-2-yl)-methanol. The alcohol may be converted to the iodide. Any conventional method of converting an alcohol to an iodide may be utilized to effect this conversion. The alkyl halide may in turn be used for the alkylation step as described above.

[0063] If it is desired to produce a compound of formula VIII or IX in which -CH<R^3 is 2(R)-tetrahydrofuran, the commercially available starting material, (R)-(+)-tetrahydro-2-furic acid, may be used. In this reaction sequence, the acid may be reduced to the corresponding alcohol. Any conventional method of converting a carboxylic acid to an alcohol may be utilized to effect this conversion. The resulting alcohol can then be converted to the corresponding triflate for the alkylation step. Any conventional method of converting an alcohol to a triflate may be utilized to effect this conversion, and any conventional means of alkylation of the alpha carbon atom of a carboxylic acid or a lower alkyl ester of a carboxylic acid with an alkyl triflate may be utilized to effect this conversion.

[0064] If it is desired to produce a compound of formula VIII or IX in which -CH<R^3 is 3-tetrahydrofuran, the commercially available starting material, tetrahydro-3-

furanmethanol, may be used. In this reaction sequence, the alcohol can first be converted to the corresponding tosylate. Any conventional method of converting an alcohol to a tosylate may be utilized to effect this conversion. The resulting tosylate may then be converted to the corresponding iodide. Any conventional method of converting a tosylate to an iodide may be utilized to effect this conversion. The subsequent alkylation reaction may then be carried out by any conventional means of alkylation of the alpha carbon atom of a carboxylic acid or a lower alkyl ester of a carboxylic acid.

[0065] If it is desired to produce a compound of formula VIII or IX in which -CH<R^3 is 2-tetrahydropyran, the commercially available starting material, 2-bromomethyl-tetrahydro-pyran, may be used as the alkyl halide. The alkylation reaction may then be carried out by any conventional means of alkylation of the alpha carbon atom of a carboxylic acid or a lower alkyl ester of a carboxylic acid.

[0066] If it is desired to produce a compound of formula VIII or IX in which -CH<R^3 is 3(R)-tetrahydrothiopyran, the commercially available starting material, 3,3'-thiodipropionate, may be used. In this reaction sequence, the diester can be cyclized using base. Any conventional method of base-promoted cyclization may be utilized to effect this conversion. The resulting thiopyran may be enzymatically reduced. Any conventional method of chiral reduction may be utilized to effect this conversion. The resulting alcohol can be reduced to the corresponding hydrocarbon. Any conventional method of alcohol reduction may be utilized to effect this conversion. The remaining ester may then be reduced to the alcohol. Any conventional method of converting an ester to an alcohol may be utilized to effect this conversion. The alcohol can then be converted to the alkyl iodide. Any conventional method of converting an alcohol to an iodide may be utilized to effect this conversion. The alkylation reaction may then be carried out by any conventional means of alkylation of the alpha carbon atom of a carboxylic acid or a lower alkyl ester of a carboxylic acid.

[0067] If it is desired to produce a compound of formula I in which -CH<R^3 is 3-(1-oxo-hexahydro-1λ^4-thiopyran-3(R)-yl, the starting material may be of the form of

formula I containing a 3(R)-tetrahydro-thiopyran subunit at -CH<R^3. The thio-ether can be oxidized to the sulfoxide. Any conventional method of oxidizing alkyl thio substituents to sulfoxides can be utilized to effect this conversion.

[0068] If it is desired to produce a compound of formula VIII or IX in which -CH<R^3 is 4-tetrahydropyran, the known starting material, (tetrahydro-pyran-4-yl)-methanol, may be used. In this reaction sequence, the alcohol may be converted to the tosylate. Any conventional method of converting an alcohol to a tosylate may be utilized to effect this conversion. The tosylate is then converted to the iodide. Any conventional method of converting an alcohol to an iodide may be utilized to effect this conversion. The alkylation reaction may then be carried out by any conventional means of alkylation of the alpha carbon atom of a carboxylic acid or a lower alkyl ester of a carboxylic acid.

[0069] If it is desired to produce a compound of formula VIII or IX in which -CH<R^3 is 2-hydroxy-cyclopentyl, the commercially available starting material, 2-oxo-cyclopetanecarboxylic acid ethyl ester, may be used. In this reaction sequence, the ketone may be reduced to the alcohol. Any conventional method of reducing a ketone to an alcohol may be utilized to effect this conversion. The resulting alcohol can then be protected using a standard protecting group for an alcohol. Any conventional method of converting an alcohol to a protected alcohol may be utilized to effect this conversion. The ester may be reduced to the corresponding primary alcohol. Any conventional method of reducing an ester to an alcohol may be utilized to effect this conversion. The resulting alcohol can be converted to the iodide. Any conventional method of converting an alcohol to an iodide may be utilized to effect this conversion. The alkylation reaction may then be carried out by any conventional means of alkylation of the alpha carbon atom of a carboxylic acid or a lower alkyl ester of a carboxylic acid. Alternatively, if it is desired to produce a compound of formula I in which -CH<R^3 is 2-oxo-cyclopentyl, the starting material may be of the form of formula I containing a 2-hydroxy-cyclopentyl subunit at -CH<R^3. The alcohol may be oxidized to the ketone. Any conventional method of oxidizing an alcohol to a ketone may be utilized to effect this conversion.

In an analogous manner:

(a) The desired 3-oxo-cyclohexyl -CH<R^3 group can be obtained from ethyl 3-oxocyclohexane-1-carboxylate.

(b) The desired 2-oxo-cyclohexyl -CH<R^3 group can be obtained from 2-cyclohexanone carboxylate.

[0070] If it is desired to produce a compound of formula I in which -CH<R^3 is 2-hydroxyimino-cyclopentyl, the starting material may be of the form of formula I containing a 2-oxo-cyclopentyl subunit at -CH<R^3. The ketone may then be converted to the hydroxyimino. Any conventional method of converting a ketone to a hydroxyimino may be utilized to effect this conversion. On the otherhand, if it is desired to produce a compound of formula I in which -CH<R^3 is 2-methoxyimino-cyclopentyl, the starting material may also be of the form of formula I containing the 2-oxo-cyclopentyl subunit at -CH<R^3. The ketone may be converted to the methoxyimino. Any conventional method of converting a ketone to a methoxyimino may be utilized to effect this conversion.

[0071] If it is desired to produce a compound of formula VIII in which -CH<R^3 is 2,2-difluoro-cyclopentyl, the starting material may be of the form of formula VIII containing a 2-oxo-cyclopentyl subunit at -CH<R^3. The ketone may then be converted to the difluoro. Any conventional method of converting a ketone to a difluoro may be utilized to effect this conversion.

[0072] If it is desired to produce a compound of formula I in which -CH<R^3 is 3-hydroxy-cyclopentyl, the starting material, 3-iodomethyl-cyclopentanone (*J. Org. Chem.* **1981**, *46*, 2412-2414), may be used. The ketone can be reduced to the alcohol. Any conventional method of reducing a ketone to an alcohol may be utilized to effect this conversion. The alcohol may be protected using a standard protecting group for an alcohol. Any conventional method of converting an alcohol to a protected alcohol may be utilized to effect this conversion. The alkylation reaction may then be carried out by any conventional means of alkylation of the alpha carbon atom of a carboxylic acid or a lower alkyl ester of a carboxylic acid. The compound of formula VIII is condensed with the compound

of formula X *via* conventional methods to produce the compound of formula I. In carrying out this reaction, any conventional method of condensing a primary amine with a carboxylic acid ester can be utilized to effect this conversion. The alcohol protecting group may then be removed. Any conventional method to remove alcohol protecting groups may be used.

[0073] If it is desired to produce a compound of formula I in which -CH<R^3 is 3-methoxy-cyclopentyl, the starting material may be of the form of formula I containing a 3-hydroxy-cyclopentyl subunit at -CH<R^3. The alcohol can be converted to a methyl ether. Any conventional method of converting an alcohol to a methyl ether may be utilized to effect this conversion. On the otherhand, if it is desired to produce a compound of formula I in which -CH<R^3 is 3-acetoxy-cyclopentyl, the starting material may also be of the form of formula I containing the 3-hydroxy-cyclopentyl subunit at -CH<R^3. The alcohol can be converted to an acetoxy group. Any conventional method of converting an alcohol to an acetoxy may be utilized to effect this conversion.

[0074] If it is desired to produce a compound of formula VIII in which -CH<R^3 is 3-fluoro-cyclopentyl, the starting material may be of the form of formula VIII containing a 3-hydroxy-cyclopentyl subunit at -CH<R^3. The alcohol can be converted to a fluoro. Any conventional method of converting an alcohol to a fluoro may be utilized to effect this conversion.

[0075] If it is desired to produce a compound of formula VIII or IX in which -CH<R^3 is 3-oxo-cyclopentyl, the starting material 3-iodomethyl-cyclopentanone (*J. Org. Chem.* **1981**, *46*, 2412-2414) may be used. The ketone can be protected using a standard protecting group for a ketone. Any conventional method of converting a ketone to a protected ketone may be utilized to effect this conversion. The alkylation reaction may then be carried out by any conventional means of alkylation of the alpha carbon atom of a carboxylic acid or a lower alkyl ester of a carboxylic acid. The protecting group of the ketone can then be removed. Any conventional method of removing a ketone protecting group may be utilized to effect this conversion. If it desired to produce a compound in which the

stereochemistry at the branch point off of the 3-oxo-cyclopentyl ring is defined as either R or S, the starting material may be the appropriately chiral-protected form of 2-cyclopentene-1-one. This material can then be converted to the appropriately protected iodide by standard methods.

[0076] If it is desired to produce a compound of formula I in which -CH<R^3 is 3-hydroxyimino-cyclopentyl, the starting material may be of the form of formula I containing a 3-oxo-cyclopentyl subunit at -CH<R^3. The ketone may be converted to the hydroxyimino. Any conventional method of converting a ketone to a hydroxyimino may be utilized to effect this conversion. On the otherhand, if it is desired to produce a compound of formula I in which -CH<R^3 is 3-methoxyimino-cyclopentyl, the starting material may also be of the form of formula I containing the 3-oxo-cyclopentyl subunit at -CH<R^3. The ketone may be converted to the methoxyimino. Any conventional method of converting a ketone to a methoxyimino may be utilized to effect this conversion.

[0077] If it is desired to produce a compound of formula VIII in which -CH<R^3 is 3,3-difluoro-cyclopentyl, the starting material may be of the form of formula VIII containing a 3-oxo-cyclopentyl subunit at -CH<R^3. The ketone may then be converted to the difluoro. Any conventional method of converting a ketone to a difluoro may be utilized to effect this conversion.

[0078] If it is desired to produce a compound of formula I in which -CH<R^3 is 3-hydroxy-3-methyl-cyclopentyl, the starting material may be of the form of formula I containing a 3-oxo-cyclopentyl subunit at -CH<R^3. The ketone may then be converted to the 3-hydroxy-3-methyl compound by any conventional method of converting a ketone to a lower alkyl tertiary alcohol.

[0079] If it is desired to produce a compound of formula VIII or IX in which -CH<R^3 is 4-oxo-cyclohexyl, the commercially available starting material, 4-cyclohexanonecarboxylic acid ethyl ester, may be used. The ketone may be protected using a standard protecting group. Any conventional method of protecting a ketone may be utilized to effect this conversion. The resulting ester

may then be reduced to the alcohol. Any conventional method of reducing an ester to an alcohol may be utilized to effect this conversion. The alcohol can be converted to the iodide. Any conventional method of converting an alcohol to an iodide may be utilized to effect this conversion. The alkylation reaction may then be carried out by any conventional means of alkylation of the alpha carbon atom of a carboxylic acid or a lower alkyl ester of a carboxylic acid. The ketone protecting group may then be removed. Any conventional method to remove a ketone protecting group may be used.

[0080] If it is desired to produce a compound of formula I in which -CH<R^3 is 4-hydroxyimino-cyclohexyl, the starting material may be of the form of formula I containing a 4-oxo-cyclohexyl subunit at -CH<R^3. The ketone may then be converted to the hydroxyimino. Any conventional method of converting a ketone to a hydroxyimino may be utilized to effect this conversion. On the otherhand, if it is desired to produce a compound of formula I in which -CH<R^3 is 4-methoxyimino-cyclohexyl, the starting material may also be of the form of formula I containing the 4-oxo-cyclohexyl subunit at -CH<R^3. The ketone may then be converted to the methoxyimino. Any conventional method of converting a ketone to a methoxyimino may be utilized to effect this conversion.

[0081] If it is desired to produce a compound of formula I in which -CH<R^3 is 4-hydroxy-cyclohexyl, the starting material may be of the form of formula I containing a 4-oxo-cyclohexyl subunit at -CH<R^3. The ketone may be reduced to the alcohol. Any conventional method of converting a ketone to a alcohol may be utilized to effect this conversion.

[0082] If it is desired to produce a compound of formula VIII or IX in which -CH<R^3 is 3-tetrahydropyran, the commercially available starting material, dihydro-pyran-3-one, may be used. In this reaction sequence, the ketone may be reduced to the alcohol. Any conventional method of reducing a ketone to an alcohol may be utilized to effect this conversion. The alcohol is converted to the mesylate. Any conventional method of converting an alcohol to a mesylate may be utilized to effect this conversion. The mesylate may then be displaced by a cyano group. Any

conventional method of converting a mesylate to a cyano may be utilized to effect this conversion. The resulting cyano may then be converted to an acid. Any conventional method of hydrolysis of a cyano to an acid may be utilized to effect this conversion. The acid is then reduced to an alcohol. Any conventional method of reducing an acid to an alcohol may be utilized to effect this conversion. The alcohol may then be converted to an iodide. Any conventional method of converting an alcohol to an iodide may be utilized to effect this conversion. The alkylation reaction may then be carried out by any conventional means of alkylation of the alpha carbon atom of a carboxylic acid or a lower alkyl ester of a carboxylic acid.

In an analogous manner:

(a) The desired 2-tetrahydrothiofuran -CH<R^3 group can be made from 4-butyrothiolactone.

(b) The desired 3-tetrahydrothiofuran -CH<R^3 group can be made from tetrahydrothiophen-3-one.

(c) The desired 4-tetrahydrothiopyran -CH<R^3 group can be made from tetrahydrothiopyran-4-one.

[0083] If it is desired to produce a compound of formula VIII or IX in which -CH<R^3 is 2-tetrahydrothiopyran, the commercially available starting material, ethyl 2-oxothiane-3-carboxylate, may be used. In this reaction sequence, the ester may be converted to the acid. Any conventional method of converting an ester to an acid may be utilized to effect this conversion. The acid may then be decarboxylated. Any conventional method of decarboxylation may be utilized to effect this conversion. The ketone may then be reduced to the alcohol. Any conventional method of reducing a ketone to an alcohol may be utilized to effect this conversion. The alcohol is converted to the mesylate. Any conventional method of converting an alcohol to a mesylate may be utilized to effect this conversion. The mesylate may then be displaced by a cyano group. Any conventional method of converting a mesylate to a cyano may be utilized to effect this conversion. The resulting cyano may then be converted to an acid. Any conventional method of hydrolysis of a cyano to an acid may be utilized to effect this conversion. The acid is then reduced to an alcohol. Any conventional method of reducing an acid to an

alcohol may be utilized to effect this conversion. The alcohol may then be converted to an iodide. Any conventional method of converting an alcohol to an iodide may be utilized to effect this conversion. The alkylation reaction may then be carried out by any conventional means of alkylation of the alpha carbon atom of a carboxylic acid or a lower alkyl ester of a carboxylic acid.

[0084] The compound of formula I has an asymmetric carbon atom through which the group -CH_2-CH<R3 and the acid amide substituents are connected. In accordance with this invention, the preferred stereoconfiguration of this group is R.

[0085] If it is desired to produce the R or the S isomer of the compounds of formula I, these compounds can be isolated as the desired isomer by conventional chemical means. The preferred chemical mean is the use of pseudoephredrine as a chiral auxiliary for the asymmetric alkylation of the phenylacetic acids of formula V (see for example, Myers, A.G. et al. *J. Am. Chem. Soc.* **1997**, 6496). To form the desired R acids of formula IX, the compounds of formula V are first converted to the pseudoephedrine amides using 1R,2R-(-)-pseudoephedrine as the desired enantiomer of pseudoephedrine. Any conventional method of converting a carboxylic acid to a carboxamide can be utilized to effect this conversion. The pseudoephedrine amides can undergo highly diastereoselective alkylations with alkyl halides to afford the α-substituted amide products corresponding to formula IX. These highly diastereomerically enriched amides can be converted to the highly enantiomerically enriched R carboxylic acids of formula IX by conventional acidic hydrolysis methods to convert a carboxamide to a carboxylic acid. These R carboxylic acids of formula IX can be converted to the R isomers of formula I. In carrying out this reaction, any conventional method of condensing a primary amine with a carboxylic acid can be utilized to effect this conversion.

[0086] Another chemical means to produce the R or S isomer of the compounds of formula I is to react the compound of formula IX with an optically active base. Any conventional optically active base can be utilized to carry out this resolution. Among the preferred optically active bases are the optically active amine bases such as alpha-methylbenzylamine, quinine, dehydroabietylamine and alpha-

methylnaphthylamine. Any of the conventional techniques utilized in resolving organic acids with optically active organic amine bases can be utilized in carrying out this reaction. In the resolution step, the compound of formula IX is reacted with the optically active base in an inert organic solvent medium to produce salts of the optically active amine with both the R and S isomers of the compound of formula IX. In the formation of these salts, temperatures and pressure are not critical and the salt formation can take place at room temperature and atmospheric pressure. The R and S salts can be separated by any conventional method such as fractional crystallization. After crystallization, each of the salts can be converted to the respective compounds of formula IX in the R and S configuration by hydrolysis with an acid. Among the preferred acids are dilute aqueous acids, i.e., from about 0.001N to 2N aqueous acids, such as aqueous sulfuric or aqueous hydrochloric acid. The configuration of formula IX which is produced by this method of resolution is carried out throughout the entire reaction scheme to produce the desired R or S isomers of formula I.

[0087] The resolution of racemates of the compounds of the formula IX can also be achieved via the formation of corresponding diastereomeric esters or amides. These diastereomeric esters or amides can be prepared by coupling the carboxylic acids of the formula IX with a chiral alcohol or a chiral amine. This reaction can be carried out using any conventional method of coupling a carboxylic acid with an alcohol or an amine. The corresponding diastereomers of compounds of the formula IX can then be separated using any conventional separation methods. The resulting pure diastereomeric esters or amides can then be hydrolyzed to yield the corresponding pure R or S isomers. The hydrolysis reaction can be carried out using conventional known methods to hydrolyze an ester or an amide without racemization. Finally, the separation of R and S isomers can also be achieved using an enzymatic ester hydrolysis of any lower alkyl esters corresponding to the compound of the formula VIII (see for example, Ahmar, M.; Girard, C.; Bloch, R, *Tetrahedron Lett*, **1989**, 7053), which results in the formation of corresponding chiral acid and chiral ester. The ester and the acid can be separated by any conventional method of separating an acid from an ester. The configuration of formula VIII which is produced by this method of resolution is carried out

throughout the entire reaction scheme to produce the desired R or S isomers of formula I.

[0088] All of the compounds of formula I which include the compounds set forth in the Examples, activated glucokinase *in vitro* by the procedure of Biological Activity Example A. In this manner, they increase the flux of glucose metabolism, which causes increased insulin secretion. Therefore, the compounds of formula I are glucokinase activators useful for increasing insulin secretion.

[0089] The following compounds were tested and found to have excellent glucokinase activator *in vivo* activity when administered orally in accordance with the assay described in Biological Activity Example B:

N-(5-Bromo-pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(tetrahydro-pyran-4-yl)-propionamide

2-(3-Chloro-4-methanesulfonyl-phenyl)-N-[5-(N-hydroxycarbamimidoyl)-pyrazin-2-yl]-3-(tetrahydro-pyran-4-yl)-propionamide

2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-3-(3-oxo-cyclopentyl)-N-pyrazin-2-yl-propionamide

2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-3-((R)-3-oxo-cyclopentyl)-N-pyrazin-2-yl-propionamide

N-(5-Bromo-pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(3-oxo-cyclopentyl)-propionamide

N-(5-Bromo-pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(3-hydroxyimino-cyclopentyl)-propionamide

2-(3-Chloro-4-methanesulfonyl-phenyl)-3-(3-methoxyimino-cyclopentyl)-N-pyrazin-2-yl-propionamide

N-(5-Bromo-pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(3-methoxyimino-cyclopentyl)-propionamide

N-(5-Bromo-pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(4-oxo-cyclohexyl)-propionamide

N-(5-Bromo-pyrazin-2-yl)-2(R)-(3-chloro-4-methanesulfonyl-phenyl)-3-(4-oxo-cyclohexyl)-propionamide

2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-N-(5-chloro-pyrazin-2-yl)-3-(4-oxo-cyclohexyl)-propionamide

N-(5-Bromo-pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(4-hydroxyimino-cyclohexyl)-propionamide

N-(5-Bromo-pyrazin-2-yl)-2(R)-(3-chloro-4-methanesulfonyl-phenyl)-3-(4-hydroxyimino-cyclohexyl)-propionamide

2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-N-(5-chloro-pyrazin-2-yl)-3-(4-hydroxyimino-cyclohexyl)-propionamide.

[0090] On the basis of their capability of activating glucokinase, the compounds of above formula I can be used as medicaments for the treatment of type II diabetes. Therefore, as mentioned earlier, medicaments containing a compound of formula I are also an object of the present invention, as is a process for the manufacture of such medicaments, which process comprises bringing one or more compounds of formula I and, if desired, one or more other therapeutically valuable substances into a galenical administration form, e.g. by combining a compound of formula I with a pharmaceutically acceptable carrier and/or adjuvant.

[0091] The pharmaceutical compositions may be administered orally, for example in the form of tablets, coated tablets, dragées, hard or soft gelatine capsules, solutions, emulsions or suspensions. Administration can also be carried out rectally, for example using suppositories; locally or percutaneously, for example using ointments, creams, gels or solutions; or parenterally, e.g. intravenously, intramuscularly, subcutaneously, intrathecally or transdermally, using for example injectable solutions. Furthermore, administration can be carried out sublingually or as an aerosol, for example in the form of a spray. For the preparation of tablets, coated tablets, dragées or hard gelatine capsules the compounds of the present invention may be admixed with pharmaceutically inert, inorganic or organic excipients. Examples of suitable excipients for tablets, dragées or hard gelatine capsules include lactose, maize starch or derivatives thereof, talc or stearic acid or salts thereof. Suitable excipients for use with soft gelatine capsules include for example vegetable oils, waxes, fats, semi-solid or liquid polyols etc.; according to the nature of the active ingredients it may however be the case that no excipient is

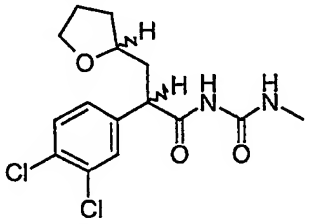
needed at all for soft gelatine capsules. For the preparation of solutions and syrups, excipients which may be used include for example water, polyols, saccharose, invert sugar and glucose. For injectable solutions, excipients which may be used include for example water, alcohols, polyols, glycerine, and vegetable oils. For suppositories, and local or percutaneous application, excipients which may be used include for example natural or hardened oils, waxes, fats and semi-solid or liquid polyols. The pharmaceutical compositions may also contain preserving agents, solubilising agents, stabilising agents, wetting agents, emulsifiers, sweeteners, colorants, odorants, salts for the variation of osmotic pressure, buffers, coating agents or antioxidants. As mentioned earlier, they may also contain other therapeutically valuable agents. It is a prerequisite that all adjuvants used in the manufacture of the preparations are non-toxic.

[0092] Preferred forms of use are intravenous, intramuscular or oral administration, most preferred is oral administration. The dosages in which the compounds of formula I are administered in effective amounts depend on the nature of the specific active ingredient, the age and the requirements of the patient and the mode of application. In general, dosages of about 1-100 mg/kg body weight per day come into consideration.

[0093] This invention will be better understood from the following examples, which are for purposes of illustration and are not intended to limit the invention defined in the claims, which follow thereafter.

Examples

Example 1
1-[2-(3,4-Dichloro-phenyl)-3-(tetrahydro-furan-2-yl)-propionyl]-3-methyl-urea



[0094] A solution of triphenylphosphine (11.90 g, 45.41 mmol) and imidazole (6.18 g, 90.82 mmol) in methylene chloride (80 mL) cooled to 0°C was slowly treated with iodine (11.53 g, 45.41 mmol) followed by the dropwise addition of a solution of (tetrahydro-furan-2-yl)-methanol (4.0 mL, 41.28 mmol) in methylene chloride (5 mL). The resulting reaction mixture was allowed to warm to 25°C, where it was stirred for 4 h. The reaction mixture was then diluted with water (25 mL), and the reaction mixture was further extracted with methylene chloride (3 x 20 mL). The combined organic layers were dried over sodium sulfate, filtered, and concentrated *in vacuo* at 25°C. The resulting solid was washed with pentane (4 x 50 mL) and filtered through a pad of silica gel. The filtrate was concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 80/20 pentane/ether) afforded 2-iodomethyl-tetrahydro-furan (2.09 g, 25%) as a clear, colorless liquid: EI-HRMS m/e calcd for C_5H_9IO (M^+) 211.9698, found 211.9708.

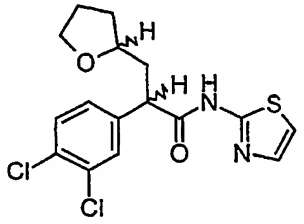
[0095] A solution of (3,4-dichloro-phenyl)-acetic acid (14.0 g, 0.07 mol) in methanol (71 mL) was treated with a catalytic amount of concentrated sulfuric acid. The reaction mixture was heated under reflux for 12 h. The reaction was concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 50/50 hexanes/ethyl acetate) afforded (3,4-dichloro-phenyl)-acetic acid methyl ester (15.0 g, quant.) as a white solid: mp 30-32°C; EI-HRMS m/e calcd for $C_9H_8Cl_2O_2$ (M^+) 217.9901, found 217.9907.

[0096] A solution of diisopropylamine (0.59 mL, 4.51 mmol) in tetrahydrofuran (30 mL) was cooled to -78°C under an argon atmosphere and then was treated with a 2.5M solution of *n*-butyllithium in hexanes (1.8 mL, 4.51 mmol). The reaction mixture was stirred at -78°C for 15 min, after which time, a solution of (3,4-

dichloro-phenyl)-acetic acid methyl ester (825 mg, 3.76 mmol) in tetrahydrofuran (3 mL) and 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (1 mL) was slowly added via a cannula. The bright yellow solution was allowed to stir at -78°C for 1 h, after which time, a solution of 2-iodomethyl-tetrahydro-furan (798 mg, 3.76 mmol) in 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (0.5 mL) was added via a cannula. The reaction mixture was stirred at -78°C for 1 h and then allowed to warm to 25°C, where it was stirred for 14 h. The reaction mixture was then quenched by the addition of a saturated aqueous ammonium chloride solution (20 mL) and extracted with ethyl acetate (3 x 20 mL). The combined organic layers were dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 90/10 hexanes/ethyl acetate) afforded 2-(3,4-dichloro-phenyl)-3-(tetrahydro-furan-2-yl)-propionic acid methyl ester (501 mg, 44%) as a colorless oil: EI-HRMS m/e calcd for $C_{14}H_{16}Cl_2O_3$ (M^+) 302.0477, found 302.0464.

[0097] A solution of 2-(3,4-dichloro-phenyl)-3-(tetrahydro-furan-2-yl)-propionic acid methyl ester (617 mg, 2.04 mmol), methylurea (302 mg, 4.07 mmol), and a solution of magnesium methoxide in methanol (7.4 wt.%, 4.63 mL, 3.06 mmol) was heated at 100°C for 8 h. After this time, the reaction mixture was concentrated *in vacuo*, dissolved in ethyl acetate (50 mL), and then filtered through a pad of silica gel. The organics were then concentrated *in vacuo* to give the crude product. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 80/20 hexanes/ethyl acetate) afforded 1-[2-(3,4-dichloro-phenyl)-3-(tetrahydro-furan-2-yl)-propionyl]-3-methyl-urea as a white solid: EI-HRMS m/e calcd for $C_{15}H_{18}Cl_2N_2O_3$ (M^+) 344.0694, found 344.0699.

Example 2
2-(3,4-Dichloro-phenyl)-3-(tetrahydro-furan-2-yl)-N-thiazol-2-yl-propionamide

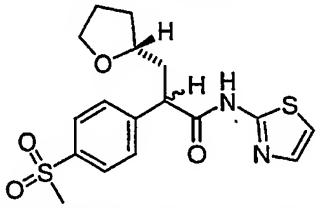


[0098] A solution of freshly prepared lithium diisopropylamide (23 mL of a 0.31M stock solution, 7.13 mmol) cooled to -78°C was treated with (3,4-dichloro-phenyl)-acetic acid (696 mg, 3.39 mmol) in tetrahydrofuran/1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (8.5 mL, 3:1). The resulting solution was stirred at -78°C for 45 min. At this time, the reaction was treated with a solution of 2-bromomethyl-tetrahydro-furan (672 mg, 4.07 mmol) in 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (2 mL). The reaction mixture was stirred at -78°C for 2 h. The reaction was then warmed to 25°C and was stirred at 25°C for 18 h. The reaction mixture was then quenched by the dropwise addition of a saturated aqueous ammonium chloride solution. The excess solvent was removed *in vacuo*. The residue was diluted with water (50 mL) and was treated with a 1N aqueous hydrochloric acid solution until the solution was acidic. The resulting solution was extracted into ethyl acetate (3 x 50 mL). The organics were washed with a saturated aqueous lithium chloride solution (1 x 100 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 50/50 hexanes/ethyl acetate with glacial acetic acid) afforded 2-(3,4-dichloro-phenyl)-3-(tetrahydro-furan-2-yl)-propionic acid (692.3 mg, 70.8%) as a white solid: mp 100-102°C; FAB-HRMS m/e calcd for $C_{13}H_{14}Cl_2O_3$ $(M+H)^+$ 289.0399, found 289.0404.

[0099] A solution of 2-(3,4-dichloro-phenyl)-3-(tetrahydro-furan-2-yl)-propionic acid (204.5 mg, 0.70 mmol), 2-aminothiazole (71 mg, 0.70 mmol), and benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (314 mg, 0.70 mmol) in *N,N*-dimethylformamide (3.55 mL) was treated with *N,N*-diisopropylethylamine (260 µL, 1.49 mmol). The mixture was stirred under nitrogen at 25°C for 18 h. At this time, the reaction was diluted with water (50 mL). This solution was extracted with ethyl acetate (3 x 50 mL). The organics were dried over sodium

sulfate, filtered, and concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 50/50 hexanes/ethyl acetate) afforded 2-(3,4-dichloro-phenyl)-3-(tetrahydro-furan-2-yl)-N-thiazol-2-yl-propionamide (232.2 mg, 88.4%) as a white solid: mp 69-71°C; EI-HRMS m/e calcd for $C_{16}H_{16}Cl_2N_2O_2S$ (M^+) 370.0309, found 370.0309.

Example 3
2-(4-Methanesulfonyl-phenyl)-3-(tetrahydro-furan-2(R)-yl)-N-thiazol-2-yl-propionamide



[0100] A solution of (R)-(+)-tetrahydro-2-furoic acid (8.0 g, 68.9 mmol) in dry tetrahydrofuran (100 mL) under argon, cooled in an ice bath, was treated dropwise with borane-dimethylsulfide (19.6 mL, 207.0 mmol). The reaction was allowed to warm to 25°C, where it was stirred for 2 h, and was then re-cooled to 0°C in an ice bath. The reaction was then quenched by the dropwise addition of water. The reaction was diluted with more water (100 mL) and extracted with ethyl acetate (3 x 75 mL). The combined organic extracts were dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 25/75 hexanes/ethyl acetate) afforded (R)-(tetrahydro-furan-2-yl)-methanol (5.91 g, 84%) as a colorless oil: $[\alpha]^{23}_{589}$ = –16.69° (c=5.2, chloroform).

[0101] A solution of (R)-(tetrahydro-thiopyran-3-yl)-methanol (1.0 g, 9.8 mmol) in methylene chloride (35 mL) was cooled to -78°C and was then treated with 2,6-lutidine (1.71 mL, 14.7 mmol) followed by trifluoromethanesulfonic anhydride (1.98 mL, 11.76 mmol). The reaction was stirred at -78°C for 40 min and was then diluted with hexanes (40 mL). The mixture was washed with a saturated aqueous sodium bicarbonate solution (1 x 25 mL). The combined organic extracts were dried over magnesium sulfate, filtered, and concentrated *in vacuo* to afford trifluoromethanesulfonic acid (R)-tetrahydro-furan-2-yl methyl ester as a crude oil.

[0102] A solution of 4-(methylthio)phenylacetic acid (6.91 g, 37.9 mmol) in methanol (100 mL) was treated slowly with concentrated sulfuric acid (1 mL). The resulting reaction mixture was heated under reflux for 19 h. The reaction mixture was allowed to cool to 25°C and then concentrated *in vacuo* to remove methanol. The resulting residue was diluted with ethyl acetate (200 mL). The organic layer was washed with a saturated aqueous sodium bicarbonate solution (3 x 300 mL) and a saturated aqueous sodium chloride solution (1 x 100 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated *in vacuo* to afford (4-methylsulfanyl-phenyl)-acetic acid methyl ester (7.28 g, 98%) as a yellow liquid which was used without further purification: EI-HRMS m/e calcd for $C_{10}H_{12}O_2S$ (M^+) 196.0558, found 196.0559.

[0103] A solution of diisopropylamine (1.21 mL, 8.62 mmol) in dry tetrahydrofuran (30 mL) was cooled to -78°C under argon and was then treated with a 2.5M solution of *n*-butyllithium in hexanes (3.3 mL, 8.25 mmol). The reaction mixture was stirred at -78°C for 30 min and then treated dropwise with a solution of (4-methylsulfanyl-phenyl)-acetic acid methyl ester (1.47 g, 7.5 mmol) in dry tetrahydrofuran (10 mL) and 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (3.34 mL). The reaction mixture turned gold in color and was allowed to stir at -78°C for 1 h. The reaction mixture was then treated with a solution of trifluoromethanesulfonic acid (R)-tetrahydro-furan-2-yl methyl ester (2.30 g, 9.8 mmol) in dry tetrahydrofuran (10 mL). The reaction mixture was allowed to warm to 25°C, where it was stirred for 16 h. The reaction mixture was quenched with a saturated aqueous ammonium chloride solution (30 mL) and then concentrated *in vacuo* to remove tetrahydrofuran. The aqueous residue was extracted with ethyl acetate (3 x 75 mL). The combined organic extracts were dried over sodium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 40M, Silica, 9/1 hexanes/ethyl acetate) afforded 2-(4-methylsulfanyl-phenyl)-3-(tetrahydro-furan-2(R)-yl)-propionic acid methyl ester (0.810 g, 39%) as a pale yellow oil: $[\alpha]^{23}_{589} = -10.87°$ (c=0.46, chloroform); EI-HRMS m/e calcd for $C_{15}H_{20}O_3S$ (M^+) 280.1133, found 280.1130.

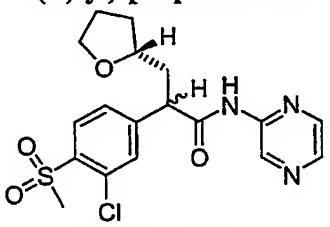
[0104] A solution of 2-(4-methylsulfanyl-phenyl)-3-(tetrahydro-furan-2(R)-yl)-propionic acid methyl ester (0.693 g, 2.47 mmol) in methanol (10 mL) was treated with a 0.8M aqueous lithium hydroxide solution (4.01 mL, 4.94 mmol). The reaction mixture was stirred at 25°C for 16 h and then was concentrated *in vacuo* to remove methanol. The remaining aqueous layer was acidified to pH=2 with a 10% aqueous hydrochloric acid solution and then was extracted with ethyl acetate (2 x 10 mL). The combined organic layers were washed with a saturated aqueous sodium chloride solution (1 x 10 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo* to afford pure 2-(4-methylsulfanyl-phenyl)-3-(tetrahydro-furan-2(R)-yl)-propionic acid (0.653 g, 99%) as a light yellow oil that crystallized upon standing: mp 105-107°C; $[\alpha]^{23}_{589} = -14.93°$ (c=0.75, chloroform); EI-HRMS m/e calcd for $C_{14}H_{18}O_3S$ (M^+) 266.0976, found 266.0976.

[0105] A solution of 2-(4-methylsulfanyl-phenyl)-3-(tetrahydro-furan-2(R)-yl)-propionic acid (0.075 g, 0.28 mmol), benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (0.186 g, 0.42 mmol), and 2-aminothiazole (0.042 g, 0.42 mmol) in methylene chloride (10 mL) at 25°C was treated with triethylamine (0.12 mL, 0.84 mmol). The resulting reaction mixture was stirred at 25°C for 16 h. The reaction mixture was then diluted with water (10 mL) and extracted with methylene chloride (3 x 10 mL). The combined organic layers were sequentially washed with water (1 x 10 mL), a 1N aqueous sodium hydroxide solution (1 x 10 mL), a 1N aqueous hydrochloric acid solution (1 x 10 mL), and a saturated aqueous sodium chloride solution (1 x 10 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 40S, Silica, 2/3 hexanes/ethyl acetate) afforded 2-(4-methanesulfanyl-phenyl)-3-(tetrahydro-furan-2(R)-yl)-N-thiazol-2-yl-propionamide (0.068 g, 70%) as a pale yellow foam: $[\alpha]^{23}_{589} = -32.91°$ (c=0.24, chloroform); EI-HRMS m/e calcd for $C_{17}H_{20}N_2O_2S_2$ (M^+) 348.0966, found 348.0968.

[0106] A solution of 2-(4-methanesulfanyl-phenyl)-3-(tetrahydro-furan-2(R)-yl)-N-thiazol-2-yl-propionamide (0.061 g, 0.18 mmol) in formic acid (0.20 mL, 5.25 mmol) was cooled to 0°C and then was treated with a 30% aqueous hydrogen

peroxide solution (0.10 mL, 0.875 mmol). The resulting solution was stirred at 0°C for 5 min and was then warmed to 25°C, where it was stirred for 1 h. The reaction was re-cooled to 0°C and was then quenched with a 10% aqueous sodium bisulfite solution. The reaction mixture was diluted with water (5 mL) and extracted with ethyl acetate (2 x 5 mL). The combined organic layers were dried over sodium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 40S, Silica, 2/3 hexanes/ethyl acetate) afforded the 2-(4-methanesulfonyl-phenyl)-3-(tetrahydro-furan-2(R)-yl)-N-thiazol-2-yl-propionamide (0.062 g, 70%) as a white foam: $[\alpha]^{23}_{589}$ = –33.0° (c=0.20, chloroform); EI-HRMS m/e calcd for $C_{17}H_{20}N_2O_4S_2$ (M^+) 380.0864, found 380.0873.

Example 4
2-(3-Chloro-4-methanesulfonyl-phenyl)-N-pyrazin-2-yl-3-(tetrahydro-furan-2(R)-yl)-propionamide



[0107] A solution of aluminum trichloride (54.9 g, 412 mmol) in chloroform (180 mL) under argon cooled to 0°C was treated dropwise with a solution of methyl chlorooxoacetate (24.3 mL, 264 mmol) in chloroform (180 mL). The reaction mixture was stirred at 0°C for 30 min and then was treated dropwise with a solution of 2-chlorothioanisole (39.4 g, 247 mmol) in chloroform (180 mL). The reaction mixture turned red in color. The resulting reaction mixture was allowed to warm to 25°C, where it was stirred for 4 h. The reaction mixture was then slowly poured onto ice (700 mL). The resulting yellow mixture was stirred for 15 min and then was filtered through celite to remove the aluminum salts. The filtrate was then extracted with methylene chloride (3 x 50 mL). The combined organic layers were washed with a saturated aqueous sodium bicarbonate solution (1 x 50 mL). The organics were then dried over magnesium sulfate, filtered, and concentrated *in vacuo* to afford (3-chloro-4-methylsulfanyl-phenyl)-oxo-acetic

acid methyl ester (36.4 g, 60%) as a light yellow oil: EI-HRMS m/e calcd for $C_{10}H_9ClO_3S$ (M^+) 243.9961, found 243.9958.

[0108] A solution of (3-chloro-4-methylsulfanyl-phenyl)-oxo-acetic acid methyl ester (61.7 g, 252 mmol) in toluene (120 mL) was heated at 50°C. This heated solution was then treated dropwise with a 3M aqueous sodium hydroxide solution (105 mL, 313 mmol) via a dropping funnel, taking care to keep the temperature below 60°C. After the addition was complete, the reaction mixture was stirred at 50°C for another 1.5 h, during which time, a yellow precipitate began to form. After this time, the heat was removed, and the warm solution was treated dropwise with concentrated hydrochloric acid (10.6 mL, 290 mmol). The resulting reaction mixture was allowed to cool to 25°C and then was stirred at 25°C for 16 h. The solid was filtered and then washed with water (50 mL) and toluene (50 mL). The solid was dried by suction for 1 h and then dried in a high vacuum desiccator to afford (3-chloro-4-methylsulfanyl-phenyl)-oxo-acetic acid (57.22 g, 98%) as a white solid: mp 166°C (dec); FAB-HRMS m/e calcd for $C_9H_7ClO_3S$ $(M+Na)^+$ 252.9702, found 252.9700.

[0109] A reaction flask equipped with mechanical stirrer was charged with hydrazine hydrate (8.5 mL, 273 mmol). The hydrazine hydrate was cooled to -50°C and then treated with (3-chloro-4-methylsulfanyl-phenyl)-oxo-acetic acid (12.6 g, 54.6 mmol) in one portion. An exotherm ensued that raised the temperature. The resulting white milky mixture was then heated to 80°C. After reaching 80°C, the heating element was removed, and the reaction mixture was then treated with potassium hydroxide (2.09 g, 31.7 mmol) in one portion. An exotherm was observed. The reaction was then stirred at 25°C until the reaction temperature cooled back to 80°C. At this time, another portion of potassium hydroxide (2.09 g, 31.7 mmol) was added. Again, an exotherm was observed, and the resulting reaction mixture was allowed to cool back to 80°C. Once at 80°C, a third portion of potassium hydroxide (2.09 g, 31.7 mmol) was added to the reaction mixture. Another exotherm was observed, and after cooling back to 80°C, the fourth and final portion of potassium hydroxide (2.09 g, 31.7 mmol) was added. At this point, the heating element was added, and the reaction mixture was heated at

100°C for 16 h. The resulting homogenous reaction mixture was cooled to 25°C and then diluted with water (12 mL). The reaction mixture was then transferred to a separatory funnel, rinsing with additional water (12 mL) and diethyl ether (40 mL). The layers were separated, and the aqueous layer was transferred to a flask. The organic layer was extracted with water (2 x 15 mL) The aqueous layers were combined and treated with heptane (20 mL), and the resulting reaction mixture was vigorously stirred. This stirred solution was then treated dropwise with concentrated hydrochloric acid (26 mL) over 30 min while the temperature was kept under 50°C with an ice bath. A cloudy suspension formed, and this suspension was stirred at 25°C for 3 h. The solid that formed was collected by filtration and then washed sequentially with a 1N aqueous hydrochloric acid solution (2 x 6 mL), heptane (1 x 12 mL), and a solution of heptane/diethyl ether (15 mL, 4:1). The resulting solid was dried under high vacuum to afford (3-chloro-4-methylsulfanyl-phenyl)-acetic acid (10.48 g, 89%) as an off-white solid: mp 105.6-108.4°C; EI-HRMS m/e calcd for $C_9H_9ClO_2S$ (M^+) 216.0012, found 216.0022.

[0110] A solution of (3-chloro-4-methylsulfanyl-phenyl)-acetic acid (7.00 g, 32.30 mmol) in methanol (150 mL) was treated slowly with concentrated sulfuric acid (2.8 mL, 52.65 mmol). The resulting reaction mixture was heated under reflux for 1.5 h. The reaction mixture was allowed to cool to 25°C and then concentrated *in vacuo* to remove methanol. The residue was diluted with ethyl acetate (500 mL). The organic phase was successively washed with a saturated aqueous sodium bicarbonate solution (1 x 200 mL) and a saturated aqueous sodium chloride solution (1 x 200 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo* to afford (3-chloro-4-methylsulfanyl-phenyl)-acetic acid methyl ester (7.41 g, 99.4%) as a light yellow oil: EI-HRMS m/e calcd for $C_{10}H_{11}ClO_2S$ (M^+) 230.0168, found 230.0166.

[0111] A solution of (R)-(tetrahydro-thiopyran-3-yl)-methanol (prepared as in Example 3, 0.418 g, 4.095 mmol) in methylene chloride (10 mL) was cooled to -78°C and was then treated with 2,4,6-collidine (830 µL, 6.3 mmol) followed by trifluoromethanesulfonic anhydride (830 µL, 4.91 mmol). The reaction was

stirred at -78°C for 40 min and was then diluted with hexanes (20 mL). The mixture was washed with a saturated aqueous sodium bicarbonate solution (1 x 10 mL), and the combined organic extracts were dried over magnesium sulfate, filtered, and concentrated *in vacuo* to afford trifluoromethanesulfonic acid (R)-tetrahydro-furan-2-yl methyl ester as a crude oil.

[0112] A solution of diisopropylamine (0.55 mL, 3.94 mmol) in dry tetrahydrofuran (10 mL) was cooled to -78°C under argon and was then treated with a 2.5M solution of *n*-butyllithium in hexanes (1.51 mL, 3.78 mmol). The reaction mixture was stirred at -78°C for 30 min and then treated dropwise with a solution of (3-chloro-4-methylsulfanyl-phenyl)-acetic acid methyl ester (0.72 g, 3.15 mmol) in dry tetrahydrofuran (3.5 mL) and 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (1.2 mL). The reaction mixture turned gold in color and was allowed to stir at -78°C for 1 h. The reaction mixture was then treated with a solution of trifluoromethanesulfonic acid (R)-tetrahydro-furan-2-yl methyl ester (0.959 g, 4.10 mmol) in dry tetrahydrofuran (2 mL). The reaction mixture was allowed to warm to 25°C, where it was stirred for 52 h. The reaction mixture was quenched with a saturated aqueous ammonium chloride solution (25 mL) and then concentrated *in vacuo* to remove tetrahydrofuran. The aqueous residue was extracted with ethyl acetate (3 x 50 mL). The combined organic extracts were dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 9/1 hexanes/ethyl acetate eluted to 85/15 hexanes/ethyl acetate) afforded 2-(3-chloro-4-methylsulfanyl-phenyl)-3-(tetrahydro-furan-2(R)-yl)-propionic acid methyl ester (0.519 g, 52%) as a pale yellow oil: $[\alpha]^{23}_{589} = -19.02°$ (c=0.51, chloroform); EI-HRMS m/e calcd for $C_{15}H_{19}ClO_3S$ (M^+) 314.0743, found 314.0743.

[0113] A solution of 2-(3-chloro-4-methylsulfanyl-phenyl)-3-(tetrahydro-furan-2(R)-yl)-propionic acid methyl ester (0.519 g, 1.65 mmol) in methanol (7 mL) was treated with a 0.8M aqueous lithium hydroxide solution (40.7 mL, 33.0 mmol). The reaction mixture was stirred at 25°C for 1.5 h and then concentrated *in vacuo* to remove methanol. The remaining aqueous layer was acidified to pH=2 with a 10% aqueous hydrochloric acid solution and then extracted with ethyl acetate (2 x

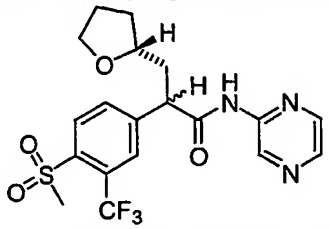
100 mL). The combined organic layers were washed with a saturated aqueous sodium chloride solution (1 x 100 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo* to afford pure 2-(3-chloro-4-methylsulfanyl-phenyl)-3-(tetrahydro-furan-2(R)-yl)-propionic acid (0.495 g, 95.8%) as a light yellow oil that crystallized upon standing: mp 93-96°C; $[\alpha]^{23}_{589}$ = –23.70° (c=0.46, chloroform); EI-HRMS m/e calcd for $C_{14}H_{17}ClO_3S$ (M^+) 300.0587, found 300.0579.

[0114] A solution of 2-(3-chloro-4-methylsulfanyl-phenyl)-3-(tetrahydro-furan-2(R)-yl)-propionic acid (60 mg, 0.2 mmol) in methylene chloride (5 mL) was treated with *N,N*-dimethylformamide (3 drops) and then cooled to 0°C. The reaction mixture was then treated with a 2.0M solution of oxalyl chloride in methylene chloride (110 µL, 0.22 mmol). The reaction mixture was stirred at 0°C for 30 min and then was allowed to warm to 25°C. The reaction was concentrated *in vacuo* to remove solvents and excess oxalyl chloride. The resulting residue was re-dissolved in dry tetrahydrofuran (5 mL) and was treated dropwise with a solution of 2-aminopyrazine (57 mg, 0.6 mmol) in tetrahydrofuran (1 mL) and pyridine (65 µL, 0.8 mmol). The resulting reaction mixture was stirred at 0°C for 45 min. The reaction mixture was then diluted with water (2 mL) and extracted with methylene chloride (3 x 5 mL). The combined organic layers were dried over sodium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 12M, Silica, 1/4 hexanes/ethyl acetate) afforded 2-(3-chloro-4-methylsulfanyl-phenyl)-N-pyrazin-2-yl-3(R)-(tetrahydro-furan-2-yl)-propionamide (50 mg, 66.1%) as a colorless gum: $[\alpha]^{23}_{589}$ = –43.33° (c=0.45, chloroform); EI-HRMS m/e calcd for $C_{18}H_{20}ClN_3O_2S$ (M^+) 377.0965, found 377.0979.

[0115] A solution of 2-(3-chloro-4-methylsulfanyl-phenyl)-N-pyrazin-2-yl-3-(tetrahydro-furan-2(R)-yl)-propionamide (0.060 g, 0.16 mmol) in formic acid (0.19 mL, 4.8 mmol) was cooled to 0°C and then treated with a 30% aqueous hydrogen peroxide solution (0.10 mL, 0.8 mmol). The resulting solution was stirred at 0°C for 30 min and was then quenched with a 10% aqueous sodium bisulfite solution. The reaction mixture was diluted with water (5 mL) and

extracted with ethyl acetate (2 x 5 mL). The combined organic layers were dried over sodium sulfate, filtered, and concentrated *in vacuo*. The resulting residue was dissolved in methanol (1 mL) and was treated dropwise with a solution of potassium permanganate (0.028 g, 0.176 mmol) in water (0.5 mL). The dark brown solution was stirred at 25°C for 30 min, and was then diluted with methanol (10 mL). The reaction mixture was filtered to remove solids, and the filtrate was concentrated *in vacuo*. Biotage chromatography (FLASH 12M, Silica, 1/1 hexanes/ethyl acetate to 1/4 hexanes/ethyl acetate) afforded the 2-(3-chloro-4-methanesulfonyl-phenyl)-N-pyrazin-2-yl-3-(tetrahydro-furan-2(R)-yl)-propionamide (44 mg, 67.1%) as a colorless gum: $C_{18}H_{20}ClN_3O_4S$ (M^+) 409.0863, found 409.0868.

Example 5
2-(4-Methanesulfonyl-3-trifluoromethyl-phenyl)-N-pyrazin-2-yl-3-(tetrahydro-furan-2(R)-yl)-propionamide



[0116] A solution of (4-fluoro-3-trifluoromethyl-phenyl)-acetic acid (4.90 g, 22.06 mmol) in methanol (40 mL) was treated with concentrated sulfuric acid (7 drops). The reaction mixture was heated under reflux for 2 h. The reaction was then concentrated *in vacuo*. The residue was dissolved in ethyl acetate (100 mL) and washed with a saturated aqueous sodium bicarbonate solution (1 x 50 mL) and a saturated aqueous sodium chloride solution (1 x 50 mL). The combined organic layers were dried over sodium sulfate, filtered, and concentrated *in vacuo* to give (4-fluoro-3-trifluoromethyl-phenyl)-acetic acid methyl ester (5.02 g, 96%) as a colorless oil.

[0117] A solution of diisopropylamine (1.86 mL, 13.29 mmol) in dry tetrahydrofuran (45 mL) cooled to -78°C under argon was treated with a 2.5M solution of *n*-butyllithium in hexanes (5.10 mL, 12.75 mmol). The reaction mixture was stirred at -78°C for 30 min and then was treated dropwise with a solution of (4-fluoro-3-

trifluoromethyl-phenyl)-acetic acid methyl ester (2.51 g, 10.63 mmol) in dry tetrahydrofuran (15 mL) and 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (6.5 mL). The reaction mixture turned gold in color and was allowed to stir at -78°C for 1 h. At this time, the reaction was treated with a solution of trifluoromethanesulfonic acid (R)-tetrahydro-furan-2-yl methyl ester (prepared as in Example 3, 3.23 g, 13.81 mmol) in dry tetrahydrofuran (15 mL). The reaction mixture was allowed to warm to 25°C, where it was stirred for 16 h. The reaction mixture was then quenched with a saturated aqueous ammonium chloride solution (30 mL) and was then concentrated *in vacuo* to remove tetrahydrofuran. The aqueous residue was extracted with ethyl acetate (3 x 150 mL). The combined organic extracts were dried over sodium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 40M, Silica, 85/15 hexanes/ethyl acetate) afforded 2-(4-fluoro-3-trifluoromethyl-phenyl)-3-(tetrahydro-furan-2(R)-yl)-propionic acid methyl ester (2.50 g, 39%) as a colorless oil: $[\alpha]^{23}_{589} = -18.62°$ (c=0.29, chloroform); EI-HRMS m/e calcd for $C_{15}H_{20}O_3S$ $(M+Na)^+$ 343.0928, found 343.0927.

[0118] A solution of 2-(4-fluoro-3-trifluoromethyl-phenyl)-3-(tetrahydro-furan-2(R)-yl)-propionic acid methyl ester (1.97 g, 6.15 mmol) in dry *N,N*-dimethylformamide (20 mL) at 25°C under argon was carefully treated with sodium thiomethoxide (0.680 g, 9.27 mmol) and was then heated at 100°C for 3 h. The reaction mixture was then concentrated *in vacuo* to remove *N,N*-dimethylformamide. The remaining residue was suspended in a saturated aqueous ammonium chloride solution (100 mL) and was extracted with ethyl acetate (2 x 200 mL). The combined organic extracts were dried over sodium sulfate, filtered, and concentrated *in vacuo*. The resulting residue was then dissolved in methanol (40 mL) and was treated with a 0.8M aqueous lithium hydroxide solution (20.60 mL, 16.7 mmol). The reaction mixture was stirred at 25°C for 16 h and then was concentrated *in vacuo* to remove methanol. The remaining aqueous layer was acidified to pH=2 with a 10% aqueous hydrochloric acid solution and then was extracted with ethyl acetate (2 x 200 mL). The combined organic layers were washed with a saturated aqueous sodium chloride solution (1 x 100 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo* to afford pure 2-(4-

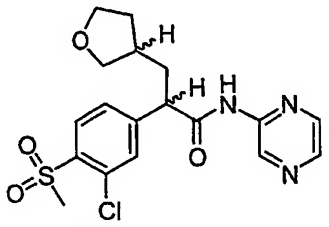
methylsulfanyl-3-trifluoromethyl-phenyl)-3-(tetrahydro-furan-2(R)-yl)-propionic acid (1.71 g, 83%) as a light yellow oil: $[\alpha]^{23}_{589} = -23.26°$ (c=0.49, chloroform); EI-HRMS m/e calcd for $C_{15}H_{17}F_3O_3S$ $(M-H_2O)^+$ 316.0744, found 316.0749.

[0119] A solution of 2-(4-methylsulfanyl-3-trifluoromethyl-phenyl)-3-(tetrahydro-furan-2(R)-yl)-propionic acid (67 mg, 0.20 mmol) in methylene chloride (5 mL) was treated with *N,N*-dimethylformamide (3 drops) and then cooled to 0°C. The reaction mixture was then treated with a 2.0M solution of oxalyl chloride in methylene chloride (0.11 mL, 0.22 mmol). The reaction mixture was stirred at 0°C for 30 min, allowed to warm to 25°C, and then was concentrated *in vacuo* to remove solvents and excess oxalyl chloride. The resulting residue was re-dissolved in dry tetrahydrofuran (5 mL) and was treated dropwise with a solution of 2-aminopyrazine (57 mg, 0.60 mmol) in tetrahydrofuran (2 mL) and pyridine (0.065 mL, 0.80 mmol). The resulting reaction mixture was stirred at 25°C for 1.5 h. The reaction mixture was then diluted with water (50 mL) and extracted with methylene chloride (3 x 50 mL). The combined organic layers were dried over sodium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 12M, Silica, 1/1 hexanes/ethyl acetate) afforded 2-(4-methylsulfanyl-3-trifluoromethyl-phenyl)-N-pyrazin-2-yl-3-(tetrahydro-furan-2(R)-yl)-propionamide (51 mg, 61%) as a colorless gum: EI-HRMS m/e calcd for $C_{19}H_{20}F_3N_3O_2S$ $(M)^+$ 411.1228, found 411.1229.

[0120] A solution of 2-(4-methylsulfanyl-3-trifluoromethyl-phenyl)-N-pyrazin-2-yl-3-(tetrahydro-furan-2(R)-yl)-propionamide (0.054 g, 0.13 mmol) in formic acid (0.16 mL, 3.0 mmol) cooled to 0°C was treated with a 30% aqueous hydrogen peroxide solution (0.08 mL, 0.65 mmol). The resulting solution was stirred at 0°C for 30 min and was then quenched with a 10% aqueous sodium bisulfite solution. The reaction mixture was diluted with water (5 mL) and extracted with ethyl acetate (2 x 5 mL). The combined organic layers were dried over sodium sulfate, filtered, and concentrated *in vacuo*. The resulting residue was dissolved in methanol (1 mL) and was treated dropwise with a solution of potassium permanganate (0.023 g, 0.143 mmol) in water (0.5 mL). The dark brown solution was stirred at 25°C for 30 min and then was diluted with methanol (10 mL). The

reaction mixture was filtered to remove solids, and the filtrate was concentrated *in vacuo*. Biotage chromatography (FLASH 12M, Silica, 7/3 hexanes/ethyl acetate to 1/1 hexanes/ethyl acetate) afforded 2-(4-methanesulfonyl-3-trifluoromethyl-phenyl)-N-pyrazin-2-yl-3-(tetrahydro-furan-2(R)-yl)-propionamide (33 mg, 57.3%) as a colorless gum: EI-HRMS m/e calcd for $C_{19}H_{20}F_3N_3O_4S$ $(M)^+$ 443.1127 found 443.1137.

Example 6
2-(3-Chloro-4-methanesulfonyl-phenyl)-N-pyrazin-2-yl-3-(tetrahydro-furan-3-yl)-propionamide



[0121] A solution of tetrahydro-3-furanmethanol (3.0 g, 29.4 mmol) in methylene chloride (45 mL) at 25°C was treated with 4-(dimethylamino)pyridine (3.99 g, 32.31 mmol) and *p*-toluenesulfonyl chloride (5.60 g, 29.37 mmol), and the reaction mixture was allowed stir at 25°C overnight. The reaction was then transferred to a separatory funnel and was then washed with a 1N aqueous hydrochloric acid solution (30 mL) and a saturated aqueous sodium bicarbonate solution (20 mL). The organic layer was then dried over sodium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 40M, Silica, 60/40 hexanes/ethyl acetate) afforded toluene-4-sulfonic acid tetrahydro-furan-3-yl methyl ester (6.57 g, 97%) as a colorless oil: $(ES)^+$-HRMS m/e calcd for $C_{12}H_{16}O_4S$.$(M+Na)^+$ 279.0661, found 279.0664.

[0122] A solution of toluene-4-sulfonic acid tetrahydro-furan-3-yl methyl ester (6.50 g, 25.36 mmol), sodium iodide (11.02 g, 73.54 mmol), and acetone (200mL) was heated to 60°C for 16 h. The resulting suspension was then cooled to 10°C and filtered. The salts were rinsed with cold acetone (50 mL). The filtrate and washings were then concentrated *in vacuo* to a thick slurry. To this slurry was added methylene chloride (100 mL), and the precipitate was filtered off and washed with methylene chloride (20 mL). The filtrate and washings were then

dried over magnesium sulfate, filtered through a pad a silica gel, and then concentrated *in vacuo* to afford 3-iodomethyl-tetrahydro-furan as a light yellow oil.

[0123] A solution of diisopropylamine (0.84 mL, 5.98 mmol) in tetrahydrofuran (10 mL) was cooled to -78°C under an argon atmosphere and then was treated with a 2.5M solution of *n*-butyllithium in hexanes (2.29 mL, 5.72 mmol). The reaction mixture was stirred at -78°C for 15 min. At this time, the reaction was slowly treated with a solution of (3-chloro-4-methylsulfanyl-phenyl)-acetic acid methyl ester (prepared as in Example 4, 1.20 g, 5.20 mmol) in tetrahydrofuran (5 mL) and 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (0.75 mL). The bright yellow solution was allowed to stir at -78°C for 1 h, after which time, a solution of 3-iodomethyl-tetrahydro-furan (2.21 g, 10.4 mmol) in 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (0.69 mL) and tetrahydrofuran (5 mL) was added via a cannula. The reaction mixture was then allowed to warm to 25°C, where it was stirred for 48 h. The reaction mixture was then quenched by the addition of a saturated aqueous ammonium chloride solution (30 mL) and extracted with ethyl acetate (3 x 30 mL). The organic layers were then combined and washed with a 10% aqueous sulfuric acid solution (25 mL) and a saturated aqueous sodium bicarbonate solution (25 mL). The combined organic layers were then dried over sodium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 40M, Silica, 75/25 hexanes/ethyl acetate) afforded 2-(3-chloro-4-methylsulfanyl-phenyl)-3-(tetrahydro-furan-3-yl-propionic acid methyl ester (663 mg, 41%) as a light yellow oil: EI-HRMS m/e calcd for $C_{15}H_{19}ClO_3S$ (M^+) 314.0743, found 314.0729.

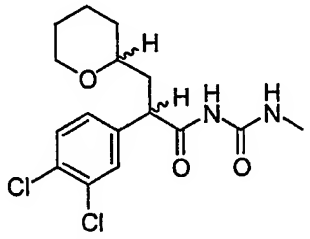
[0124] A solution of 2-(3-chloro-4-methylsulfanyl-phenyl)-3-(tetrahydro-furan-3-yl-propionic acid methyl ester (663 mg, 2.11 mmol) in formic acid (0.79 mL) and tetrahydrofuran (2.89 mL) was cooled in an ice bath to 0°C and then was treated with a 30% aqueous hydrogen peroxide solution (1.19 mL, 10.53 mmol). The reaction was then slowly warmed to 25°C and was stirred at 25°C for 16 h. At this time, the reaction was cooled to 0°C and was then quenched with a saturated aqueous sodium sulfite solution. This solution was extracted with ethyl acetate (3

x 20 mL). The combined organic layers were dried over sodium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 40S, Silica, 50/50 hexanes/ethyl acetate) afforded 2-(3-chloro-4-methanesulfonyl-phenyl)-3-(tetrahydro-furan-3-yl)-propionic acid methyl ester (729 mg, 100%) as a white waxy solid: $(ES)^+$-HRMS m/e calcd for $C_{15}H_{19}ClO_5S$ $(M+Na)^+$ 369.0534, found 369.0536.

[0125] A solution of 2-(3-chloro-4-methanesulfonyl-phenyl)-3-(tetrahydro-furan-3-yl)-propionic acid methyl ester (729 mg, 2.10 mmol) in ethanol (20 mL) was treated with a solution of potassium hydroxide (694 mg, 10.51 mmol) in water (7 mL). The reaction was stirred for 3 h at 25°C, concentrated *in vacuo* to remove the ethanol, and then was acidified to pH=2 with a 1N aqueous hydrochloric acid solution. The resulting mixture was then extracted with methylene chloride (3 x 10 mL). The organic layers were dried over sodium sulfate, filtered, and concentrated *in vacuo* to afford 2-(3-chloro-4-methanesulfonyl-phenyl)-3-(tetrahydro-furan-3-yl)-propionic acid (654 mg, 94%) as a white foam: $(ES)^+$-HRMS m/e calcd for $C_{14}H_{17}ClO_5S$ $(M+Na)^+$ 355.0377, found 355.0382.

[0126] A solution of triphenylphosphine (118 mg, 0.45 mmol) in methylene chloride (5 mL) cooled to 0°C was treated with *N*-bromosuccinimide (91 mg, 0.51 mmol). Upon complete dissolution, the cooled, purple solution was then treated with 2-(3-chloro-4-methanesulfonyl-phenyl)-3-(tetrahydro-furan-3-yl)-propionic acid (100 mg, 0.30 mmol). The resulting reaction mixture was stirred at 0°C for 20 min then warmed to 25°C, where it was stirred for another 30 min. The purple reaction mixture was then treated with 2-aminopyrazine (43 mg, 0.45 mmol) and pyridine (0.07 mL, 0.90 mmol) and stirred for 16 h at 25°C. The reaction was then diluted with water (10 mL) and extracted with methylene chloride (3 x 15 mL). The organics were dried over sodium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 12M, Silica, 1.5/98.5 methylene chloride/methanol) afforded 2-(3-chloro-4-methanesulfonyl-phenyl)-N-pyrazin-2-yl-3-(tetrahydro-furan-3-yl)-propionamide (46 mg, 37%) as a light orange foam: $(ES)^+$-HRMS m/e calcd for $C_{18}H_{20}ClN_3O_4S$ $(M+H)^+$ 410.0936, found 410.0940.

Example 7
1-[2-(3,4-Dichloro-phenyl)-3-(tetrahydro-pyran-2-yl)-propionyl]-3-methyl urea

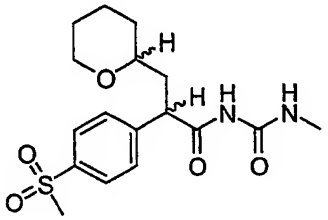


[0127] A solution of diisopropylamine (2.63 mL, 18.2 mmol) in tetrahydrofuran (120 mL) cooled to -78°C under an argon atmosphere was treated with a 2.0M solution of *n*-butyllithium in hexanes (9.1 mL, 18.2 mmol). The reaction mixture was stirred at -78°C for 15 min, after which time, a solution of (3,4-dichloro-phenyl)-acetic acid methyl ester (prepared as in Example 1, 3.62 g, 16.5 mmol) in tetrahydrofuran (20 mL) and 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (6 mL) was slowly added via a cannula. The bright yellow solution was allowed to stir at -78°C for 1 h, after which time, a solution of 2-bromomethyl-tetrahydro-pyran (2.5 mL, 19.8 mmol) in 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (4 mL) was added via a cannula. The reaction mixture was then allowed to warm to 25°C, where it was stirred for 16 h. The reaction mixture was then quenched by the addition of a saturated aqueous ammonium chloride solution (30 mL) and then extracted with ethyl acetate (3 x 40 mL). The combined organic layers were dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 90/10 hexanes/ethyl acetate) afforded 2-(3,4-dichloro-phenyl)-3-tetrahydro-pyran-2-yl)-propionic acid methyl ester (4.68 g, 89%) as a colorless oil: EI-HRMS m/e calcd for $C_{15}H_{18}Cl_2O_3$ (M^+) 316.0633, found 316.0625.

[0128] A solution of 2-(3,4-dichloro-phenyl)-3-tetrahydro-pyran-2-yl)-propionic acid methyl ester (374 mg, 1.19 mmol), methylurea (176 mg, 2.38 mmol), and a solution of magnesium methoxide in methanol (7.4 wt.%, 2.5 mL, 1.78 mmol) was heated at 100°C for 8 h. Over time, the reaction mixture turned cloudy in appearance. At this time, the reaction was concentrated *in vacuo*. The residue was dissolved in ethyl acetate (10 mL) and then filtered through a pad of silica gel. The filtrate was then concentrated *in vacuo*. Flash chromatography (Merck Silica

gel 60, 230-400 mesh, 95/5 hexanes/ethyl acetate to 60/40 hexanes/ethyl acetate) afforded the two diastereomeric pairs of 1-[2-(3,4-dichloro-phenyl)-3-(tetrahydro-pyran-2-yl)-propionyl]-3-methyl urea (76 mg, 19%) as white solids: (1) first pair of diastereomers: mp 172.8-174.2°C; EI-HRMS m/e calcd for $C_{16}H_{20}Cl_2N_2O_3$ (M^+) 358.0851, found 358.0848; (2) second pair of diastereomers: EI-HRMS m/e calcd for $C_{16}H_{20}Cl_2N_2O_3$ (M^+) 358.0851, found 358.0848.

Example 8
1-[2-(4-Methanesulfonyl-phenyl)-3-(tetrahydro-pyran-2-yl)-propionyl]-3-methyl-urea



[0129] A solution of 4-(methanesulfonyl)phenyl acetic acid (43.63 g, 0.204 mol) in methanol (509 mL) was treated slowly with concentrated sulfuric acid (2 mL). The resulting reaction mixture was heated under reflux for 19 h. The reaction mixture was allowed to cool to 25°C and then concentrated *in vacuo* to remove methanol. The residue was diluted with ethyl acetate (800 mL). The organic phase was washed with a saturated aqueous sodium bicarbonate solution (1 x 200 mL) and a saturated aqueous sodium chloride solution (1 x 200 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 70-230 mesh, 1/1 hexanes/ethyl acetate) afforded 4-(methanesulfonyl)phenyl acetic acid methyl ester (45.42 g, 98%) as a yellow oil which solidified to a cream colored solid upon sitting over time at 25°C: mp 78-80°C; EI-HRMS m/e calcd for $C_{10}H_{12}O_4S$ (M^+) 228.0456, found 228.0451.

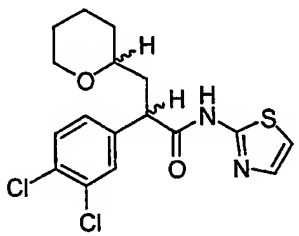
[0130] A solution of diisopropylamine (0.67 mL, 4.82 mmol) in tetrahydrofuran (30 mL) cooled to -78°C under an argon atmosphere was treated with a 2.5M solution of *n*-butyllithium in hexanes (1.93 mL, 4.82 mmol). The reaction mixture was stirred at -78°C for 15 min. At this time, the reaction was treated with a solution of (4-methanesulfonyl-phenyl)-acetic acid methyl ester (1.00 g, 4.38 mmol) in

tetrahydrofuran (6 mL) and 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (2 mL). The bright yellow solution was allowed to stir at -78°C for 1 h, after which time, a solution of 2-bromomethyl-tetrahydro-pyran (0.67 mL, 5.26 mmol) in 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (1 mL) was added via a cannula. The reaction mixture was then allowed to warm to 25°C, where it was stirred for 16 h. The reaction mixture was then quenched by the addition of a saturated aqueous ammonium chloride solution (20 mL) and extracted with ethyl acetate (3 x 15 mL). The organic layers were dried over sodium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 40M, Silica, 70/30 hexanes/ethyl acetate) afforded 2-(4-methanesulfonyl-phenyl)-3-(tetrahydro-pyran-2-yl)-propionic acid methyl ester (157 mg, 11%) as a colorless oil: EI-HRMS m/e calcd for $C_{16}H_{22}O_5S$ (M^+) 326.1188, found 326.1189.

[0131] A solution of 2-(4-methanesulfonyl-phenyl)-3-(tetrahydro-pyran-2-yl)-propionic acid methyl ester (75 mg, 0.23 mmol), methylurea (34 mg, 0.46 mmol), and a solution of magnesium methoxide in methanol (7.4 wt.%, 0.49 mL, 0.35 mmol) and methanol (0.5 mL) was heated at 100°C for 8 h. Over time, the reaction mixture turned cloudy in appearance. At this time, the reaction was concentrated *in vacuo*. The residue was dissolved in ethyl acetate (10 mL) and then filtered through a pad of silica gel. The filtrate was concentrated *in vacuo*. Biotage chromatography (FLASH 12M, Silica, 30/70 hexanes/ethyl acetate) afforded 1-[2-(4-methanesulfonyl-phenyl)-3-(tetrahydro-pyran-2-yl)-propionyl]-3-methyl-urea (5 mg, 6%) as a colorless oil: FAB-HRMS m/e calcd for $C_{17}H_{24}N_2O_5S$ $(M+H)^+$ 369.1484, found 369.1495.

Example 9

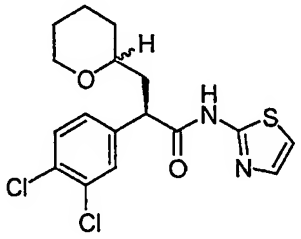
2-(3,4-Dichloro-phenyl)-3-(tetrahydro-pyran-2-yl)-N-thiazol-2-yl-propionamide



[0132] A solution of 2-(3,4-dichloro-phenyl)-3-tetrahydro-pyran-2-yl)-propionic acid methyl ester (prepared as in Example 7, 4.68 g, 14.75 mmol) in ethanol (150 mL) was treated with a solution of potassium hydroxide (1.66 g, 29.50 mmol) in water (16 mL), and the reaction was stirred for at 25°C 1 h. The reaction was then diluted with water (50 mL), concentrated *in vacuo* to remove the ethanol, and then acidified to pH=2 with a 1N aqueous hydrochloric acid solution. The product was then extracted with methylene chloride (3 x 20 mL), and the combined organic layers were dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 90/10 methylene chloride/methanol plus 1% acetic acid) afforded 2-(3,4-dichloro-phenyl)-3-(tetrahydro-pyran-2-yl)-propionic acid (3.91 g, 87%) as a clear colorless oil: EI-HRMS m/e calcd for $C_{14}H_{16}Cl_2O_3$ (M^+) 302.04765, found 302.0473.

[0133] A solution of 2-(3,4-dichloro-phenyl)-3-(tetrahydro-pyran-2-yl)-propionic acid (81 mg, 0.27 mmol) in *N,N*-dimethylformamide (5 mL) was treated with *O*-benzotriazol-1-yl-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (112 mg, 0.29 mmol), *N,N*-diisopropylethylamine (0.14 mL. 0.80 mmol), and 2-aminothiazole (40 mg, 0.40 mmol). The reaction was stirred at 25°C for 16 h. At this time, the reaction was diluted with water (10 mL) and extracted with ethyl acetate (3 x 15 mL). The combined organic layers were washed with water (1 x 10 mL), a 1N aqueous sodium hydroxide solution (1 x 10 mL), a 1N aqueous hydrochloric acid solution (1 x 10 mL), and a saturated aqueous sodium chloride solution (1 x 10 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 90/10 hexanes/ethyl acetate to 60/40 hexanes/ethyl acetate) afforded 2-(3,4-dichloro-phenyl)-3-(tetrahydro-pyran-2-yl)-N-thiazol-2-yl-propionamide (35 mg, 34%) as a light yellow oil: EI-HRMS m/e calcd for $C_{17}H_{18}Cl_2N_2O_2S$ (M^+) 384.0466, found 384.0468.

Example 10
2(R)-(3,4-Dichloro-phenyl)-3-(tetrahydro-pyran-2-yl)-N-thiazol-2-yl-propionamide

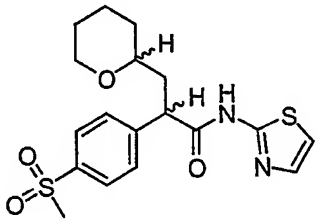


[0134] A solution of 2-(3,4-dichloro-phenyl)-3-(tetrahydro-pyran-2-yl)-propionic acid (prepared as in Example 9, 2.56 g, 8.44 mmol) in tetrahydrofuran (80 mL) cooled to -78°C was treated with triethylamine (1.30 mL, 9.65 mmol) followed by trimethylacetyl chloride (1.10 mL, 8.84 mmol). The resulting white slurry was stirred at -78°C for 15 min and then at 0°C for 45 min. In a separate flask, a solution of (S)-4-isopropyl-2-oxazolidinone (1.04 g, 8.04 mmol) in tetrahydrofuran (40 mL) cooled to -78°C was treated with a 2.5M solution of *n*-butyllithium in hexanes (3.4 mL, 8.44 mmol). The solution was stirred at -78°C for 10 min and then was allowed to warm to 25°C, where it was stirred for an additional 10 min. At this time, the first reaction mixture was re-cooled to -78°C. The second reaction mixture was added to the first reaction mixture over a period of 5 min via a cannula. The combined reaction mixture was then stirred at -78°C for 15 min and then was allowed to warm to 25°C, where it was stirred for an additional 1.5 h. At this time, the reaction was quenched by the addition of a saturated aqueous sodium bisulfite solution (25 mL) and then was extracted with ethyl acetate (3 x 30 mL). The combined organic layers were washed with a saturated aqueous sodium bicarbonate solution (1 x 15 mL) and a saturated aqueous sodium chloride solution (1 x15 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, hexanes to 80/20 hexanes/ethyl acetate) afforded two products: (1) 3-[2(S)-(3,4-dichloro-phenyl)-3-(tetrahydro-pyran-2-yl)-propionyl]-4(S)-isopropyl-oxazolidin-2-one (506 mg, 15%) as a clear colorless oil; and (2) 3-[2(R)-(3,4-dichloro-phenyl)-3-(tetrahydro-pyran-2-yl)-propionyl]-4(S)-isopropyl-oxazolidin-2-one (560 mg, 17%) as a clear colorless oil.

[0135] A solution of 3-[2(R)-(3,4-dichloro-phenyl)-3-(tetrahydro-pyran-2-yl)-propionyl]-4(S)-isopropyl-oxazolidin-2-one (560 mg, 1.40 mmol) in tetrahydrofuran (30 mL) and water (10 mL) cooled to 0°C was treated with a 30% aqueous hydrogen peroxide solution (0.7 mL) and lithium hydroxide (117 mg, 2.80 mmol). The reaction was stirred at 0°C for 1 h. At this time, the reaction was quenched with an aqueous sodium sulfite solution (0.71 g, 5.6 mmol in 4 mL) followed by the addition of a 0.5N aqueous sodium bicarbonate solution (13 mL). The tetrahydrofuran was then removed *in vacuo*. The residue was diluted with water (60 mL) and extracted with methylene chloride (3 x 20 mL). The aqueous layer was then acidified to pH=2 with a 5N aqueous hydrochloric acid solution and then was extracted with ethyl acetate (4 x 25 mL). The combined organic layers were then dried over sodium sulfate, filtered, and concentrated *in vacuo* to afford 2(R)-(3,4-dichloro-phenyl)-3-(tetrahydro-pyran-2-yl)-propionic acid (203 mg, 48%) as clear colorless oil.

[0136] A solution of 2(R)-(3,4-dichloro-phenyl)-3-(tetrahydro-pyran-2-yl)-propionic acid (80 mg, 0.26 mmol), benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluoro-phosphate (177 mg, 0.40 mmol), and 2-aminothiazole (40 mg, 0.40 mmol) in methylene chloride (10 mL) at 25°C was treated with triethylamine (0.11 mL, 0.79 mmol). The resulting reaction mixture was stirred at 25°C for 16 h. The reaction mixture was then diluted with water (10 mL) and extracted with methylene chloride (3 x 10 mL). The combined organic layers were sequentially washed with water (1 x 10 mL), a 1N aqueous sodium hydroxide solution (1 x 10 mL), a 1N aqueous hydrochloric acid solution (1 x 10 mL), and a saturated sodium chloride solution (1 x 10 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 95/5 hexanes/ethyl acetate to 70/30 hexanes/ethyl acetate) afforded the 2(R)-(3,4-dichloro-phenyl)-3-(tetrahydro-pyran-2-yl)-N-thiazol-2-yl-propionamide (57 mg, 57%) as a white foam: EI-HRMS m/e calcd for $C_{17}H_{18}Cl_2N_2O_2S$ (M^+) 384.066, found 384.1467.

Example 11
2-(4-Methanesulfonyl-phenyl)-3-(tetrahydro-pyran-2-yl)-N-thiazol-2-yl-propionamide

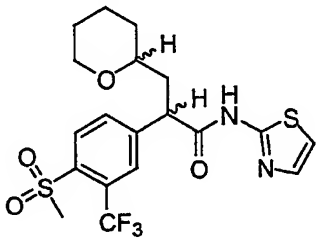


[0137] A solution of 2-(4-methanesulfonyl-phenyl)-3-(tetrahydro-pyran-2-yl)-propionic acid methyl ester (prepared as in Example 8, 75 mg, 0.23 mmol) in ethanol (5 mL) was treated with a solution of potassium hydroxide (32 mg, 0.58 mmol) in water (1 mL). The reaction was stirred for 3 h at 25°C. The reaction was then concentrated *in vacuo* to remove the ethanol and then acidified to pH=2 with a 1N aqueous hydrochloric acid solution. This solution was then extracted with methylene chloride (3 x 15 mL). The combined organic layers were dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, hexanes to 60/40 hexanes/ethyl acetate plus 1% acetic acid) afforded 2-(4-methanesulfonyl-phenyl)-3-(tetrahydro-pyran-2-yl)-propionic acid (60 mg, 85%) as a white solid: FAB-HRMS m/e calcd for $C_{15}H_{20}O_5S$ $(M+H)^+$ 313.1109, found 313.1111.

[0138] A solution of 2-(4-methanesulfonyl-phenyl)-3-(tetrahydro-pyran-2-yl)-propionic acid (60 mg, 0.19 mmol), benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluoro-phosphate (127 mg, 0.29 mmol), and 2-aminothiazole (28 mg, 0.29 mmol) in methylene chloride (5 mL) at 25°C was treated with triethylamine (0.08 mL, 0.58 mmol). The resulting reaction mixture was stirred at 25°C for 16 h. The reaction mixture was then diluted with water (10 mL) and extracted with methylene chloride (3 x 15 mL). The combined organic layers were sequentially washed with water (1 x 10 mL), a 1N aqueous sodium hydroxide solution (1 x 10 mL), a 1N aqueous hydrochloric acid solution (1 x 10 mL), and a saturated sodium chloride solution (1 x 10 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 12M, Silica, 20/80 hexanes/ethyl acetate) afforded 2-(4-

methanesulfonyl-phenyl)-3-(tetrahydro-pyran-2-yl)-N-thiazol-2-yl-propionamide (54 mg, 71%) as a colorless oil: EI-HRMS m/e calcd for $C_{18}H_{22}N_2O_4S_2$ (M^+) 394.1021, found 394.1021.

Example 12
2-(4-Methylsulfonyl-3-trifluoromethyl-phenyl)-3-(tetrahydro-pyran-2-yl)-N-thiazole-2-yl-propionamide



[0139] A solution of (tetrahydro-pyran-2-yl)-methanol (3.40 g, 29.26 mmol) in dry methylene chloride (140 mL) and 2,6-lutidine (5.23 mL, 45.02 mmol) was cooled to -78°C under argon and then treated with trifluoromethanesulfonic anhydride (5.78 mL, 35.11 mmol). The reaction mixture was stirred at -78°C for 1 h and then diluted with hexanes (200 mL). The mixture was then washed with a 50% aqueous sodium bicarbonate solution (150 mL), dried over magnesium sulfate, and concentrated *in vacuo* to afford trifluoromethanesulfonic acid tetrahydro-pyran-2-yl methyl ester as a crude oil which was used without further purification.

[0140] A solution of diisopropylamine (7.04 mL, 49.97 mmol) in dry tetrahydrofuran (140 mL) cooled to -78°C under argon was treated with a 2.5M solution of *n*-butyllithium in hexanes (19.8 mL, 49.5 mmol). The reaction mixture was stirred at -78°C for 30 min and then treated dropwise with a solution of (4-fluoro-3-trifluoromethyl-phenyl)-acetic acid (5.00 g, 22.51 mmol) in dry tetrahydrofuran (25 mL) and 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (8.5 mL). The reaction mixture turned gold in color and was allowed to stir at -78°C for 1 h. At this time, the reaction was treated with a solution of trifluoromethanesulfonic acid tetrahydro-pyran-2-yl methyl ester (7.26 g, 29.26 mmol) in dry tetrahydrofuran (100 mL). The reaction mixture was allowed to warm to 25°C, where it was stirred for 30 min. The reaction mixture then was quenched with a saturated aqueous ammonium chloride solution (100 mL) and then concentrated *in vacuo* to

remove tetrahydrofuran. The aqueous residue was acidified to pH=2 using a 1N aqueous hydrochloric acid solution. The resulting aqueous layer was extracted with ethyl acetate (2 x 250 mL). The combined organic extracts were dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 3/7 hexanes/ethyl acetate to 1/1 hexanes/ethyl acetate) afforded 2-(4-fluoro-3-trifluoromethyl-phenyl)-3-(tetrahydro-pyran-2-yl)-propionic acid (5.20 g, 72%) as a yellow gum: EI-HRMS m/e calcd for $C_{15}H_{16}F_4O_3$ $(M+Na)^+$ 343.0931, found 343.0928.

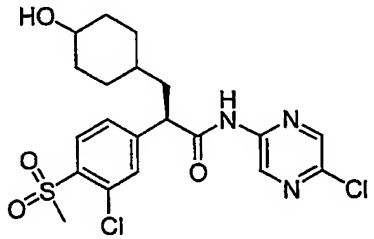
[0141] A solution of 2-(4-fluoro-3-trifluoromethyl-phenyl)-3-(tetrahydro-pyran-2-yl)-propionic acid (5.20 g, 16.24 mmol) in dry *N,N*-dimethylformamide (90 mL) at 25°C under argon was carefully treated with 95% sodium hydride (410 mg, 17.05 mmol). The reaction mixture was stirred at 25°C for 30 min and then was treated with sodium thiomethoxide (2.40 g, 32.48 mmol). This mixture was heated at 100°C for 4.5 h and then concentrated *in vacuo* to remove *N,N*-dimethylformamide. The residue was diluted with water (100 mL) and then was acidified to pH=2 using a 1N aqueous hydrochloric acid solution. The resulting aqueous layer was extracted with ethyl acetate (2 x 600 mL). The combined organic extracts were dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 98/2 chloroform/methanol to 9/1 chloroform/methanol) afforded 2-(4-methylsulfanyl-3-trifluoromethyl-phenyl)-3-(tetrahydro-pyran-2-yl)-propionic acid (5.70 g, 100%) as a yellow gum: EI-HRMS m/e calcd for $C_{16}H_{19}F_3O_3S$ $(M+Na)^+$ 371.0899, found 371.0902.

[0142] A solution of 2-(4-methylsulfanyl-3-trifluoromethyl-phenyl)-3-(tetrahydro-pyran-2-yl)-propionic acid (348 mg, 1.0 mmol) in formic acid (1.20 mL, 30 mmol) and tetrahydrofuran (1.0 mL) cooled to 0°C was treated with a 30% aqueous hydrogen peroxide solution (1.34 mL, 10 mmol). The resulting solution was allowed to warm to 25°C, where it was stirred for 24 h. The reaction was then re-cooled to 0°C, quenched with a saturated aqueous sodium bisulfite solution, and then extracted with ethyl acetate (2 x 50 mL). The combined organic layers were dried over sodium sulfate, filtered, and concentrated *in vacuo* to afford 2-(4-

methylsulfonyl-3-trifluoromethyl-phenyl)-3-(tetrahydro-pyran-2-yl)-propionic acid (378 mg, 100%) as a colorless gum: EI-HRMS m/e calcd for $C_{16}H_{19}F_3O_5S$ $(M+Na)^+$ 403.0797 found 403.0803.

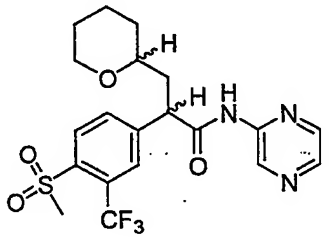
[0143] A solution of 2-(4-methylsulfonyl-3-trifluoromethyl-phenyl)-3-(tetrahydro-pyran-2-yl)-propionic acid (50 mg, 0.13 mmol) in methylene chloride (1 mL) was treated with *N,N*-dimethylformamide (3 drops) and then cooled to 0°C. The reaction mixture was then treated with a 2.0M solution of oxalyl chloride in methylene chloride (0.08 mL, 0.156 mmol). The reaction mixture was stirred at 0°C for 30 min, allowed to warm to 25°C, and then concentrated *in vacuo* to remove solvents and excess oxalyl chloride. The resulting residue was re-dissolved in dry tetrahydrofuran (1 mL) and was treated dropwise with a solution of 2-aminothiazole (28 mg, 0.27 mmol) in tetrahydrofuran (1 mL) and 2,6-lutidine (0.08 mL, 0.65 mmol). The resulting reaction mixture was stirred at 25°C for 16 h. The reaction mixture was then diluted with water (50 mL) and extracted with methylene chloride (3 x 50 mL). The combined organic layers were dried over sodium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 12M, Silica, 7/3 hexanes/ethyl acetate to 6/4 hexanes/ethyl acetate to 1/1 hexanes/ethyl acetate) afforded 2-(4-methylsulfonyl-3-trifluoromethyl-phenyl)-3-(tetrahydro-pyran-2-yl)-N-thiazole-2-yl-propionamide (37 mg, 61%) as a white foam: EI-HRMS m/e calcd for $C_{19}H_{21}F_3N_2O_4S_2$ $(M+H)^+$ 463.0968 found 463.0974.

Example 13
2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-N-(5-chloro-pyrazin-2-yl)-3-(4-hydroxy-cyclohexyl)-propionamide



[0144] A solution of 2(R)-(3-chloro-4-methanesulfonyl-phenyl)-N-(5-chloro-pyrazin-2-yl)-3-(4-oxo-cyclohexyl)-propionamide (prepared as in Example 61, 30.0 mg, 0.064 mmol) in methanol (0.5 mL) was treated with sodium borohydride (6.03 mg, 0.16 mmol). The reaction mixture was stirred at 25°C for 5 min. The reaction was then diluted with ethyl acetate (5 mL) and quenched by the dropwise addition of water. The reaction was then diluted with more water (5 mL) and concentrated *in vacuo* to remove methanol. The resulting slurry was extracted with ethyl acetate (3 x 5 mL), and the combined organic extracts were dried over magnesium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 12S, Silica, 3/7 hexanes/ethyl acetate) afforded 2(R)-(3-chloro-4-methanesulfonyl-phenyl)-N-(5-chloro-pyrazin-2-yl)-3-(4-hydroxy-cyclohexyl)-propionamide (29.0 mg, 96.3%) as a light yellow foam: EI-HRMS m/e calcd for $C_{20}H_{23}Cl_2N_3O_4S$ $(M+H)^+$ 472.0859, found 472.0866.

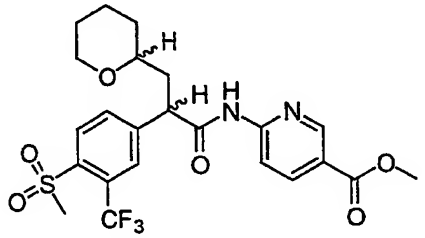
Example 14
2-(4-Methanesulfonyl-3-trifluoromethyl-phenyl)-N-pyrazin-2-yl-3-(tetrahydro-pyran-2-yl)-propionamide



[0145] A solution of 2-(4-methylsulfonyl-3-trifluoromethyl-phenyl)-3-(tetrahydro-pyran-2-yl)-propionic acid (prepared as in Example 12, 50 mg, 0.13 mmol) in methylene chloride (1 mL) was treated with *N,N*-dimethylformamide (3 drops) and then cooled to 0°C. The reaction mixture was then treated with a 2.0M solution of oxalyl chloride in methylene chloride (0.08 mL, 0.156 mmol). The reaction mixture was stirred at 0°C for 30 min, allowed to warm to 25°C, and then concentrated *in vacuo* to remove solvents and excess oxalyl chloride. The resulting residue was re-dissolved in dry tetrahydrofuran (1 mL) and was treated dropwise with a solution of 2-aminopyrazine (15 mg, 0156 mmol) in tetrahydrofuran (1 mL) and 2,6-lutidine (0.02 mL, 0.157 mmol). The resulting reaction mixture was stirred at 25°C for 16 h. The reaction mixture was then

diluted with water (50 mL) and extracted with methylene chloride (3 x 50 mL). The combined organic layers were dried over sodium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 12M, Silica, 7/3 hexanes/ethyl acetate to 1/1 hexanes/ethyl acetate) afforded 2-(4-methanesulfonyl-3-trifluoromethyl-phenyl)-N-pyrazin-2-yl-3-(tetrahydro-pyran-2-yl)-propionamide (13 mg, 22%) as a colorless gum: EI-HRMS m/e calcd for $C_{20}H_{22}F_3N_3O_4S$ $(M+Na)^+$ 480.1175 found 480.1177.

Example 15
6-[2-(4-Methylsulfonyl-3-trifluoromethyl-phenyl)-3-(tetrahydro-pyran-2-yl)-propionylamino]-nicotinic acid methyl ester

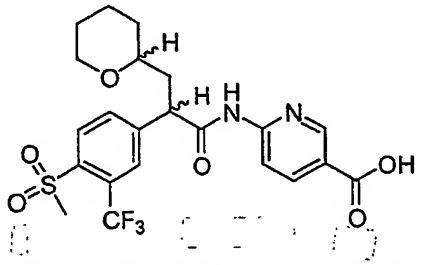


[0146] A mixture of 6-aminonicotinic acid (4.0 g, 28.9 mmol), methanol (75 mL), and concentrated hydrochloric acid (4 mL) was heated under reflux for 16 h. The reaction mixture was allowed to cool to 25°C and then concentrated *in vacuo* to remove methanol. The resulting solid was treated with water (20 mL) and enough sodium bicarbonate to adjust the pH to pH=8. The solution was then extracted with ethyl acetate (3 x 25 mL). The combined organic layers were dried over sodium sulfate, filtered, and concentrated *in vacuo* to afford 6-aminonicotinic acid methyl ester (3.12 g, 71%) as white foam: EI-HRMS m/e calcd for $C_7H_8N_2O_2$ (M^+) 152.0586, found 152.0586.

[0147] A solution of 2-(4-methylsulfonyl-3-trifluoromethyl-phenyl)-3-(tetrahydro-pyran-2-yl)-propionic acid (prepared as in Example 12, 300 mg, 0.79 mmol) in methylene chloride (6 mL) was treated with *N,N*-dimethylformamide (7 drops) and then cooled to 0°C. The reaction mixture was then treated with a 2.0M solution of oxalyl chloride in methylene chloride (0.48 mL, 0.95 mmol). The reaction mixture was stirred at 0°C for 30 min, allowed to warm to 25°C, and then concentrated *in vacuo* to remove solvents and excess oxalyl chloride. The

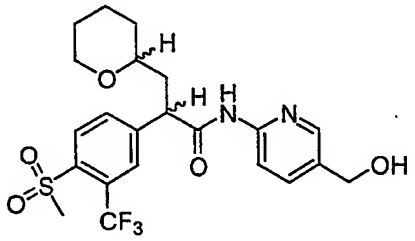
resulting residue was re-dissolved in dry tetrahydrofuran (6 mL) and was treated dropwise with a solution of 6-aminonicotinic acid methyl ester (252 mg, 1.67 mmol) in tetrahydrofuran (5 mL) and 2,6-lutidine (0.48 mL, 3.95 mmol). The resulting reaction mixture was stirred at 25°C for 16 h. The reaction mixture was then diluted with water (100 mL) and extracted with methylene chloride (3 x 50 mL). The combined organic layers were dried over sodium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 40S, Silica, 7/3 hexanes/ethyl acetate to 1/1 hexanes/ethyl acetate) afforded 6-[2-(4-methylsulfonyl-3-trifluoromethyl-phenyl)-3-(tetrahydro-pyran-2-yl)-propionylamino]-nicotinic acid methyl ester (227 mg, 55%) as a white foam: EI-HRMS m/e calcd for $C_{23}H_{25}F_3N_2O_6S$ $(M+H)^+$ 537.1277 found 537.1284.

Example 16
6-[2-(4-Methylsulfonyl-3-trifluoromethyl-phenyl)-3-(tetrahydro-pyran-2-yl)-propionylamino]-nicotinic acid



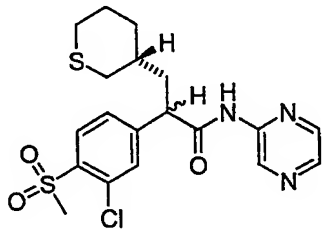
[0148] A solution of 6-[2-(4-methylsulfonyl-3-trifluoromethyl-phenyl)-3-(tetrahydro-pyran-2-yl)-propionylamino]-nicotinic acid methyl ester (prepared as in Example 15, 21 mg, 0.04 mmol) in methanol (0.40 mL) was treated with a 1N aqueous sodium hydroxide solution (0.20 mL, 0.20 mmol). The reaction mixture was stirred at 25°C for 1 h. The reaction mixture was then diluted with water and concentrated *in vacuo* to remove methanol. The resulting aqueous residue was acidified to pH=2 with a 1N aqueous hydrochloric acid solution and then extracted with ethyl acetate (2 x 10 mL). The combined organic layers were dried over sodium sulfate, filtered, and concentrated *in vacuo* to afford pure 6-[2-(4-methylsulfonyl-3-trifluoromethyl-phenyl)-3-(tetrahydro-pyran-2-yl)-propionylamino]-nicotinic acid (15 mg, 75%) as a colorless gum: EI-HRMS m/e calcd for $C_{22}H_{23}F_3O_6S$ $(M+H)^+$ 501.1302, found 501.1305.

Example 17
N-(5-Hydroxymethyl-pyridin-2-yl)-2-(4-methylsulfonyl-3-trifluoromethyl-phenyl)-3-(tetrahydro-pyran-2-yl)-propionamide



[0149] A solution of 6-[2-(4-methylsulfonyl-3-trifluoromethyl-phenyl)-3-(tetrahydro-pyran-2-yl)-propionylamino]-nicotinic acid methyl ester (prepared as in Example 15, 129 mg, 0.25 mmol) in ethyl ether (6 mL) cooled to 0°C was treated with a 1.0M solution of lithium aluminum hydride solution in diethyl ether (0.30 mL, 0.30 mmol). The orange-colored reaction mixture was stirred at 0°C for 1 h. The reaction was then quenched by the dropwise addition of water (10 mL) and extracted with ethyl acetate (2 x 10 mL). The combined organic extracts were dried over sodium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 40S Silica, 8/2 hexanes/ethyl acetate) afforded N-(5-hydroxymethyl-pyridin-2-yl)-2-(4-methanesulfonyl-3-trifluoromethyl-phenyl)-3-(tetrahydro-pyran-2-yl)-propionamide (44 mg, 36%) as a cream foam: EI-HRMS m/e calcd for $C_{22}H_{25}F_3N_2O_5S$ $(M+H)^+$ 487.1509, found 487.1514.

Example 18
2-(3-Chloro-4-methanesulfonyl-phenyl)-N-pyrazin-2-yl-3-(tetrahydro-thiopyran-3(R)-yl)-propionamide



[0150] A slurry of (3-chloro-4-methylsulfanyl-phenyl)-acetic acid methyl ester (prepared as in Example 4, 1.00 g, 4.33 mmol) in formic acid (6.54 mL, 92.30 mmol) was cooled to 0°C and then was treated with a 30% aqueous hydrogen peroxide solution (1.47 mL, 13.85 mmol). The resulting solution was allowed to

warm to 25°C, where it was stirred for 15 h. The reaction was cooled in an ice bath, and the product was precipitated by the addition of water (100 mL). The solid was filtered off, washed with water, and dried by suction to afford pure (3-chloro-4-methanesulfonyl-phenyl)-acetic acid methyl ester (1.11 g, 97.5%) as a white solid: mp 54-57°C; EI-HRMS m/e calcd for $C_{10}H_{11}ClO_4S$ (M^+) 262.0066, found 262.0060.

[0151] A solution of dimethyl 3,3'-thiodipropionate (30.00 g, 144.0 mmol) in ethylene glycol dimethyl ether (200 mL), under nitrogen, was treated with sodium hydride (7.00 g, 145.8 mmol), and the reaction was heated under reflux for 1.5 h. The reaction mixture was then cooled to 25°C and was slowly treated with a saturated aqueous sodium bicarbonate solution. The solvent was then removed *in vacuo*, and the resulting residue was diluted with water (500 mL) and extracted with ethyl acetate (3 x 300 mL). The combined organic extracts were then washed with a saturated aqueous sodium bicarbonate solution (1 x 150 mL) followed by a saturated aqueous sodium chloride solution (1 x 150 mL). The organic layer was dried over magnesium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography, performed in two batches, (FLASH 40M, Silica, 9/1 hexanes/diethyl ether) afforded 4-hydroxy-5,6-dihydro-2H-thiopyran-3-carboxylic acid methyl ester (12.89 g, 51.4%) as a colorless oil.

[0152] A suspension of baker's yeast (Saf-Instant yeast, 30 g) in water (700 mL) was treated with sucrose (142 g). The mixture was stirred at 25°C for 6 h. At this time, 4-hydroxy-5,6-dihydro-2H-thiopyran-3-carboxylic acid methyl ester (4.06 g, 23.31 mmol) was added. The resulting slurry was stirred at 25°C for 22 h and then was filtered through a pad of celite. The filtrate was extracted with ethyl acetate (5 x 300 mL). The combined organic extracts were washed with a saturated aqueous sodium chloride solution (1 x 150 mL), dried over magnesium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 40M, Silica, 3/1 hexanes/ethyl acetate) afforded 4(S)-hydroxy-tetrahydro-thiopyran-3(R)-carboxylic acid methyl ester (4.08 g, 99.3%) as a colorless oil: $[\alpha]^{23}_{589} = +37.76°$ (c=2.9, methanol)

[0153] A solution of 4(S)-hydroxy-tetrahydro-thiopyran-3(R)-carboxylic acid methyl ester (1.00 g, 5.674 mmol) in dry tetrahydrofuran (50 mL), under argon, was treated with 1,1'-thiocarbonyldiimidazole (2.085 g, 11.35 mmol) and pyridine (6.88 μL, 8.511 mmol). The reaction mixture was stirred at 25°C for 36 h and was then diluted with ethyl acetate (100 mL). The resulting solution was washed sequentially with a saturated aqueous sodium bicarbonate solution (1 x 50 mL) and a saturated aqueous sodium chloride solution (1 x 50 mL). The combined organic extracts were dried over magnesium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 40M, Silica, 4/1 hexanes/ethyl acetate) afforded 4(S)-(imidazole-1-carbothioyloxy)-tetrahydro-thiopyran-3(R)-carboxylic acid methyl ester (901 mg, 55.4%) as a beige oil: EI-HRMS m/e calcd for $C_{11}H_{14}N_2O_3S_2$ (M^+) 286.0446, found 286.0438.

[0154] A solution of tributyltin hydride (1.702 mL, 6.292 mmol) in dioxane (82.59 mL) was treated with 2,2'-azobisisobutyronitrile (110.7 mg, 0.661 mmol). The resulting mixture was heated under reflux for 2 h. The reaction was then treated with a solution of 4(S)-(imidazole-1-carbothioyloxy)-tetrahydro-thiopyran-3(R)-carboxylic acid methyl ester (901 mg, 3.146 mmol) in dioxane (3 mL). The reaction was heated under reflux for an additional 30 min and then was cooled in an ice bath. The solvent was removed *in vacuo*. The resulting residue was suspended in acetonitrile (100 mL) and was washed with hexanes (3 x 30 mL). The resulting solution was concentrated *in vacuo*, dissolved in dry tetrahydrofuran (18.07 mL) under argon, cooled in an ice bath, and then treated with a 1.0M solution of lithium aluminum hydride in tetrahydrofuran (3.159 mL, 3.159 mmol). The reaction was stirred at 0°C for 20 min and was then quenched by the addition of ethyl acetate (30 mL). The reaction mixture was then treated with a saturated aqueous ammonium chloride solution, and the layers were separated. The aqueous layer was then washed with ethyl acetate (2 x 25 mL), and the combined organic extracts were washed with a saturated aqueous sodium chloride solution, dried over magnesium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 12M, Silica, 7/3 hexanes/ethyl acetate) afforded (R)-(tetrahydro-thiopyran-3-yl)-methanol (291 mg, 69.7%) as a colorless oil: $[\alpha]^{23}_{589}$ =

–4.87° (c=0.78, methanol); EI-HRMS m/e calcd for $C_6H_{12}OS$ (M^+) 132.0609, found 132.0614.

[0155] A solution of (R)-(tetrahydro-thiopyran-3-yl)-methanol (180 mg, 1.36 mmol) in methylene chloride (16.82 mL) cooled to -78°C was treated with 2,6-lutidine (192.8 µL, 1.66 mmol) followed by trifluoromethanesulfonic anhydride (268.0 µL, 1.63 mmol). The reaction was stirred at -78°C for 10 min and was then diluted with hexanes (20 mL). The mixture was washed with a saturated aqueous sodium bicarbonate solution (1 x 10 mL). The combined organic extracts were dried over magnesium sulfate, filtered, and concentrated *in vacuo*. The resulting residue was dissolved in acetone (17 mL) and was treated with sodium iodide (612.3 mg, 4.086 mmol). The reaction mixture was stirred at 25°C for 10 min and then concentrated *in vacuo*. The residue was suspended in methylene chloride (25 mL). This mixture was washed with water (1 x 15 mL), dried over magnesium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 12S, Silica, 97/3 hexanes/ethyl acetate) afforded 3(R)-iodomethyl-tetrahydro-thiopyran (139 mg, 42.2%) as a light yellow oil which was not characterized but was used immediately for the alkylation reaction.

[0156] A 2.0M solution of lithium diisopropylamide in heptane/tetrahydrofuran/ethylbenzene (564.0 µL, 1.128 mmol) and *N,N,N',N'*-tetramethylethylenediamine (157.0 µL, 1.040 mmol) in dry tetrahydrofuran (3 mL) cooled to -78°C under nitrogen was treated with (3-chloro-4-methanesulfonyl-phenyl)-acetic acid methyl ester (269.4 mg, 1.02 mmol). The reaction mixture was stirred at -78°C for 1 h and then warmed to 0°C. The reaction was then treated with a solution of 3(R)-iodomethyl-tetrahydro-thiopyran (71.00 mg, 0.293 mmol) in dry tetrahydrofuran (1 mL) and allowed to warm to 25°C, where it was stirred for 19 h. The reaction mixture was then quenched with a saturated aqueous ammonium chloride solution (10 mL) and then concentrated *in vacuo* to remove tetrahydrofuran. The aqueous residue was extracted with ethyl acetate (2 x 20 mL), and the combined organic extracts were dried over magnesium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 12M, Silica, 4/1 hexanes/ethyl acetate) afforded 2-(3-chloro-4-methanesulfonyl-phenyl)-

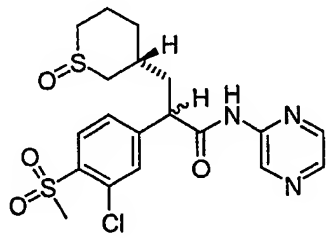
3(R)-(tetrahydro-thiopyran-3-yl)-propionic acid methyl ester (96 mg, 86.9%) as a colorless oil: $[\alpha]^{23}_{589}$ = +10.93° (c=0.76, methanol); EI-HRMS m/e calcd for $C_{16}H_{21}ClO_4S_2$ (M^+) 376.0570, found 376.0574.

[0157] A solution of 2-(3-chloro-4-methanesulfonyl-phenyl)-3(R)-(tetrahydro-thiopyran-3-yl)-propionic acid methyl ester (247 mg, 0.655 mmol) in methanol (4.75 mL) was treated with a 0.8M aqueous lithium hydroxide solution (7.27 mL, 5.895 mmol). The reaction mixture was stirred at 25°C for 1.5 h and then concentrated *in vacuo* to remove methanol. The remaining aqueous layer was acidified to pH=2 with a 10% aqueous hydrochloric acid solution and then extracted with ethyl acetate (2 x 100 mL). The combined organic layers were washed with a saturated aqueous sodium chloride solution (1 x 100 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo* to afford pure 2-(3-chloro-4-methanesulfonyl-phenyl)-3(R)-(tetrahydro-thiopyran-3-yl)-propionic acid (227.0 mg, 95.5%) as a light yellow foam: $[\alpha]^{23}_{589}$ = +12.21° (c=0.79, methanol).

[0158] A solution of 2-(3-chloro-4-methanesulfonyl-phenyl)-3(R)-(tetrahydro-thiopyran-3-yl)-propionic acid (52 mg, 0.143 mmol) in methylene chloride (1 mL) was treated with *N,N*-dimethylformamide (0.1 mL) and then cooled to 0°C. The reaction mixture was treated with a 2.0M solution of oxalyl chloride in methylene chloride (143 µL, 0.286 mmol). The reaction mixture was stirred at 0°C for 30 min, allowed to warm to 25°C, and then concentrated *in vacuo* to remove solvents and excess oxalyl chloride. The resulting residue was re-dissolved in dry tetrahydrofuran (1 mL) and was treated dropwise with a solution of 2-aminopyrazine (27.2 mg, 0.286 mmol) in tetrahydrofuran (1 mL) and pyridine (57.8 µL, 0.715 mmol). The resulting reaction mixture was stirred at 25°C for 30 min. The reaction mixture was then diluted with water (2 mL) and extracted with methylene chloride (3 x 5 mL). The combined organic layers were dried over sodium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 12M, Silica, 1/1 hexanes/ethyl acetate) afforded 2-(3-chloro-4-methanesulfonyl-phenyl)-N-pyrazin-2-yl-3(R)-(tetrahydro-thiopyran-3-yl)-

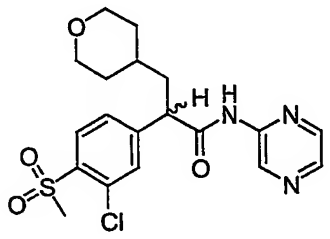
propionamide (44 mg, 69.8%) as a colorless gum: EI-HRMS m/e calcd for $C_{19}H_{22}ClN_3O_3S_2$ $(M+H)^+$ 440.0864, found 440.0867.

Example 19
2-(3-Chloro-4-methanesulfonyl-phenyl)-3-(1-oxo-hexahydro-1λ⁴-thiopyran-3(R)-yl)-N-pyrazin-2-yl-propionamide



[0159] A slurry of 2-(3-chloro-4-methanesulfonyl-phenyl)-N-pyrazin-2-yl-3-(tetrahydro-thiopyran-3(R)-yl)-propionamide (prepared as in Example 18, 10.0 mg, 0.0227 mmol) in formic acid (68.5 μL, 1.816 mmol) cooled to 0°C was treated with a 30% aqueous hydrogen peroxide solution (7.7 μL, 0.068). The resulting solution was stirred at 0°C for 10 min. The reaction was quenched by the addition of a 10% aqueous sodium sulfite solution (1 mL) and then extracted with ethyl acetate (2 x 5 mL). The organics were dried over magnesium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 12S, Silica, 93/7 chloroform/methanol) afforded 2-(3-chloro-4-methanesulfonyl-phenyl)-3-(1-oxo-hexahydro-1λ⁴-thiopyran-3(R)-yl)-N-pyrazin-2-yl-propionamide (10.0 mg, 96.5%) as a beige gum: EI-HRMS m/e calcd for $C_{19}H_{22}ClN_3O_4S_2$ $(M+Na)^+$ 478.0632, found 478.0632.

Example 20
2-(3-Chloro-4-methanesulfonyl-phenyl)-N-pyrazin-2-yl-3-(tetrahydro-pyran-4-yl)-propionamide



[0160] A solution of (tetrahydro-pyran-4-yl)-methanol (1.0 g, 8.61 mmol, prepared according to WO 99/00385) in methylene chloride (30 mL) at 25°C was treated with 4-(dimethylamino)pyridine (1.17 g, 9.47 mmol) and *p*-toluenesulfonyl chloride (1.64 g, 8.61 mmol) and then was allowed to stir at 25°C overnight. The reaction was then transferred to a separatory funnel and washed with a 1N aqueous hydrochloric acid solution (10 mL), a saturated aqueous sodium bicarbonate solution (10 mL), and a saturated aqueous sodium chloride solution (10 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 40S, Silica, 75/25 hexanes/ethyl acetate) afforded toluene-4-sulfonic acid tetrahydro-pyran-4-yl methyl ester (1.77 g, 76%) as a colorless oil.

[0161] A solution of toluene-4-sulfonic acid tetrahydro-pyran-4-yl methyl ester (1.77 g, 6.55 mmol) and sodium iodide (2.85 g, 18.99 mmol) in acetone (26 mL) was heated to 60°C for 16 h. The resulting suspension was then cooled to 10°C and filtered. The salts were rinsed with cold acetone (5 mL), and the filtrate and washings were concentrated *in vacuo* to a thick slurry. This slurry was treated with methylene chloride (10 mL). The resulting precipitate was removed by filtration and was washed with methylene chloride (10 mL). The filtrate and washings were then dried over magnesium sulfate, filtered through a pad of silica gel, and then concentrated *in vacuo* to afford 4-iodomethyl-tetrahydro-pyran as a light yellow oil.

[0162] A solution of diisopropylamine (0.33 mL, 2.38 mmol) in tetrahydrofuran (6 mL) cooled to -78°C under an argon atmosphere was treated with a 2.5M solution of *n*-butyllithium in hexanes (0.95 mL, 2.38 mmol). The reaction mixture was stirred at -78°C for 15 min, after which time, a solution of (3-chloro-4-methylsulfanyl-phenyl)-acetic acid methyl ester (prepared as in Example 4, 500 mg, 2.17 mmol) in tetrahydrofuran (1 mL) and 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (0.5 mL) was slowly added via a cannula. The greenish yellow solution was allowed to stir at -78°C for 1 h, after which time, a solution of 4-iodomethyl-tetrahydro-pyran (588 mg, 2.60 mmol) in 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (0.5 mL) was added via a cannula. The reaction mixture was then allowed to warm to 25°C, where it was stirred for 16 h. The

reaction mixture was then quenched by the addition of a saturated aqueous ammonium chloride solution (30 mL). This solution was extracted with ethyl acetate (3 x 20 mL). The combined organic layers were washed with a 10% aqueous sulfuric acid solution (2 x 50 mL) and a saturated aqueous sodium bicarbonate solution (2 x 50 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 40S, Silica, 75/25 hexanes/ethyl acetate) afforded 2-(3-chloro-4-methylsulfanyl-phenyl)-3-(tetrahydro-pyran-4-yl)-propionic acid methyl ester (431 mg, 61%) as a yellow oil: EI-HRMS m/e calcd for $C_{16}H_{21}ClO_3S$ (M^+) 328.0900, found 328.0898.

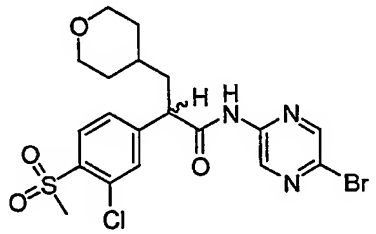
[0163] A solution of 2-(3-chloro-4-methylsulfanyl-phenyl)-3-(tetrahydro-pyran-4-yl)-propionic acid methyl ester (200 mg, 0.61 mmol) in formic acid (0.23 mL) and tetrahydrofuran (0.5 mL) cooled to 0°C was treated with a 30% aqueous hydrogen peroxide solution (0.35 mL, 3.04 mmol). The reaction was slowly warmed to 25°C where it was stirred for 16 h. The reaction mixture was then cooled to 0°C, quenched with a saturated aqueous sodium sulfite solution, and then extracted with ethyl acetate (3 x 20 mL). The organics were dried over sodium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 12M, Silica, 60/40 hexanes/ethyl acetate) afforded 2-(3-chloro-4-methanesulfonyl-phenyl)-3-(tetrahydro-pyran-4-yl)-propionic acid methyl ester (190 mg, 87%) as a colorless oil: $(ES)^+$-HRMS m/e calcd for $C_{16}H_{21}ClO_5S$ $(M+Na)^+$ 383.0690, found 383.0692.

[0164] A solution of 2-(3-chloro-4-methanesulfonyl-phenyl)-3-(tetrahydro-pyran-4-yl)-propionic acid methyl ester (190 mg, 0.53 mmol) in ethanol (10 mL) was treated with a solution of potassium hydroxide (174 mg, 2.64 mmol) in water (4 mL). The reaction was stirred at 25°C for 2.5 h. The reaction was then concentrated *in vacuo* to remove the ethanol and then acidified to pH=2 with a 1N aqueous hydrochloric acid solution. The resulting solution was then extracted with methylene chloride (3 x 10 mL). The combined organic layers were dried over sodium sulfate, filtered, and concentrated *in vacuo* to afford 2-(3-chloro-4-methanesulfonyl-phenyl)-3-(tetrahydro-pyran-4-yl)-propionic acid (167 mg, 92%)

as a white foam: $(ES)^+$-HRMS m/e calcd for $C_{15}H_{19}ClO_5S$ $(M+Na)^+$ 369.0534, found 369.0536.

[0165] A solution of 2-(3-chloro-4-methanesulfonyl-phenyl)-3-(tetrahydro-pyran-4-yl)-propionic acid (165 mg, 0.48 mmol) in methylene chloride (12 mL) and *N,N*-dimethylformamide (1 drop) cooled to 0°C was treated dropwise with a 2.0M solution of oxalyl chloride in methylene chloride (0.27 mL, 0.55 mmol). The reaction was stirred at 0°C for 30 min. At this time, the reaction was concentrated *in vacuo* to yield a light yellow oil. This oil was dissolved in tetrahydrofuran (5 mL) and then treated with a solution of 2-aminopyrazine (91 mg, 0.95 mmol) dissolved in tetrahydrofuran (10 mL) and pyridine (0.19 mL, 2.4 mmol). The reaction was then stirred at 25°C for 16 h. At this time, the reaction was diluted with water (15 mL) and extracted with methylene chloride (3 x 25 mL). The combined organic layers were dried over sodium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 12M, Silica, 25/75 hexanes/ethyl acetate) afforded 2-(3-chloro-4-methanesulfonyl-phenyl)-N-pyrazin-2-yl-3-(tetrahydro-pyran-4-yl)-propionamide (100 mg, 50%) as a white foam: $(ES)^+$-HRMS m/e calcd for $C_{19}H_{22}ClN_3O_4S$ $(M+H)^+$ 424.1093, found 424.1095.

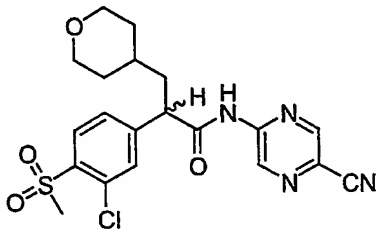
Example 21
N-(5-Bromo-pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(tetrahydro-pyran-4-yl)-propionamide



[0166] A solution of 2-(3-chloro-4-methanesulfonyl-phenyl)-3-(tetrahydro-pyran-4-yl)-propionic acid (prepared as in Example 20, 470 mg, 1.36 mmol) in methylene chloride (25 mL) and *N,N*-dimethylformamide (1 drop) cooled to 0°C was treated with a 2.0M solution of oxalyl chloride in methylene chloride (0.78 mL, 1.56 mmol) and then was stirred at 0°C for 30 min. At this time, the reaction was concentrated *in vacuo* to yield a light yellow oil. This oil was dissolved in

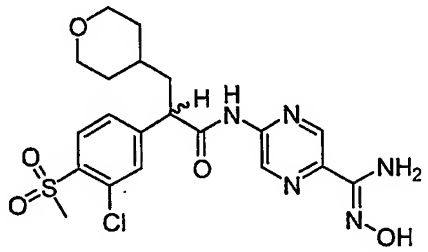
tetrahydrofuran (10 mL) and then treated with a solution of 2-amino-5-bromopyrazine (472 mg, 2.71 mmol, prepared according to *Tetrahedron* **1988**, *44*, 2977-2983) in tetrahydrofuran (20 mL) and pyridine (0.55 mL, 6.78 mmol). The reaction was then stirred at 25°C for 16 h. At this time, the reaction was diluted with water (15 mL) and extracted with methylene chloride (3 x 25 mL). The organics were dried over sodium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 40S, Silica, 40/60 hexanes/ethyl acetate) afforded N-(5-bromo-pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(tetrahydro-pyran-4-yl)-propionamide (477 mg, 70%) as a yellow foam: $(ES)^+$-HRMS m/e calcd for $C_{19}H_{21}ClBrN_3O_4S$ $(M+H)^+$ 502.0198, found 502.0205.

Example 22
2-(3-Chloro-4-methanesulfonyl-phenyl)-N-(5-cyano-pyrazin-2-yl)-3-(tetrahydro-pyran-4-yl)-propionamide



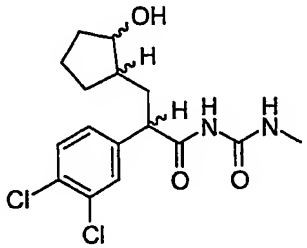
[0167] A solution of N-(5-bromo-pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(tetrahydro-pyran-4-yl)-propionamide (prepared as in Example 21, 325 mg, 0.65 mmol) in *N,N*-dimethylformamide (5 mL) at 25°C was treated with potassium cyanide (105 mg, 1.62 mmol), copper(I) iodide (307 mg, 1.62 mmol), tetrakis(triphenylphosphine)palladium(0) (22 mg, 0.02 mmol), and 18-crown-6 (25 mg, 0.09 mmol). This reaction mixture was then heated to 150°C under argon for 5 h. At this time, the reaction was cooled to 25°C, concentrated to half the volume, and then chloroform (40 mL) was added until all the salts precipitated out of solution. The salts were removed by filtration through a pad of celite and washed with chloroform. The filtrate was then concentrated *in vacuo*. Biotage chromatography (FLASH 40S, Silica, 30/70 hexanes/ethyl acetate) afforded 2-(3-chloro-4-methanesulfonyl-phenyl)-N-(5-cyano-pyrazin-2-yl)-3-(tetrahydro-pyran-4-yl)-propionamide (219 mg, 76%) as a white foam: $(ES)^+$-HRMS m/e calcd for $C_{20}H_{21}ClN_4O_4S$ $(M+H)^+$ 449.1045, found 449.1046.

Example 23
2-(3-Chloro-4-methanesulfonyl-phenyl)-N-[5-(N-hydroxycarbamimidoyl)-pyrazin-2-yl]-3-(tetrahydro-pyran-4-yl)-propionamide



[0168] A solution of 2-(3-chloro-4-methanesulfonyl-phenyl)-N-(5-cyano-pyrazin-2-yl)-3-(tetrahydro-pyran-4-yl)-propionamide (prepared as in Example 22, 151 mg, 0.34 mmol) in ethanol (2 mL) and water (1 mL) was treated with hydroxylamine hydrochloride (28 mg, 0.40 mmol) and sodium carbonate (35 mg, 0.34 mmol). The reaction was heated at 70°C for 3 h. At this time, the reaction was concentrated *in vacuo* and then extracted with 10% methanol/chloroform (3 x 30 mL). The organics were dried over magnesium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 12M, Silica, 20/80 hexanes/ethyl acetate) afforded 2-(3-chloro-4-methanesulfonyl-phenyl)-N-[5-(N-hydroxycarbamimidoyl)-pyrazin-2-yl]-3-(tetrahydro-pyran-4-yl)-propionamide (110 mg, 68%) as a white solid: mp 137.2–140.5°C; $(ES)^+$-HRMS m/e calcd for $C_{20}H_{24}ClN_5O_5S$ $(M+H)^+$ 482.1260, found 482.1263.

Example 24
1-[2-(3,4-Dichloro-phenyl)-3-(2-hydroxy-cyclopentyl)-propionyl]-3-methyl-urea



[0169] A solution of 2-oxo-cyclopentanecarboxylic acid ethyl ester (10 g, 64.0 mmol) in ethanol (106.7 mL) cooled to 0°C was treated with 98% sodium borohydride (686 mg, 17.78 mmol). The reaction was stirred at 0°C for 30 min. At this time,

the reaction mixture was poured into water (53 mL) and was extracted into diethyl ether (3 x 100 mL). The organics were dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 75/25 ethyl acetate/hexanes) afforded 2-hydroxy-cyclopentanecarboxylic acid ethyl ester (8.5 g, 83.9%) as a clear liquid.

[0170] A solution of 2-hydroxy-cyclopentanecarboxylic acid ethyl ester (3.5 g, 22.12 mmol) in methylene chloride (147.5 mL) was treated with 3,4-dihydro-2*H*-pyran (3.03 mL, 33.1 mmol) and pyridinium *p*-toluenesulfonate (556 mg, 2.21 mmol). This solution was stirred at 25°C for 5 h. The reaction was then washed with a half-saturated aqueous sodium chloride solution (2 x 75 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 90/10 hexanes/ethyl acetate) afforded 2-(tetrahydro-pyran-2-yloxy)-cyclopentanecarboxylic acid ethyl ester (4.7 g, 87.7%) as a clear liquid: EI-HRMS m/e calcd for $C_{13}H_{22}O_4$ (M^+) 242.1518 found 242.1521.

[0171] A slurry of lithium aluminum hydride (883 mg, 23.27 mmol) in tetrahydrofuran (19.4 mL) cooled to 0°C was treated with 2-(tetrahydro-pyran-2-yloxy)-cyclopentanecarboxylic acid ethyl ester (4.7 g, 19.59 mmol). The reaction was stirred at 25°C for 18 h. At this time, the reaction was poured onto ice/water. This mixture was filtered through a pad of celite (methylene chloride as eluent). The organics were washed with a saturated aqueous sodium chloride solution (1 x 100 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo* to give [2-(tetrahydro-pyran-2-yloxy)-cyclopentyl]-methanol (3.25 g, 83.6%) as a clear liquid: EI-HRMS m/e calcd for $C_{11}H_{20}O_3$ (M^+) 200.1412 found 200.1412.

[0172] A solution of triphenylphosphine (1.70 g, 6.49 mmol) and imidazole (884 mg, 12.98 mmol) in methylene chloride (8.32 mL) cooled to 0°C was treated with iodine (1.64 g, 6.49 mmol). After the iodine was completely dissolved, a solution of [2-(tetrahydro-pyran-2-yloxy)-cyclopentyl]-methanol (1.0 g, 4.99 mmol) was added to the reaction mixture. The reaction was stirred at 0°C for 1 h and at 25°C for 2 h. At this time, the reaction was poured into water (100 mL) and extracted with methylene chloride (1 x 30 mL). The organics were washed with a saturated

aqueous sodium thiosulfate solution (1 x 50 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo* at 25°C. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 80/20 hexanes/ethyl acetate) afforded 2-(2-iodomethyl-cyclopentyloxy)-tetrahydropyran (1.17 g, 75.8%) as a clear liquid: EI-HRMS m/e calcd for $C_{11}H_{19}IO_2$ (M^+) 309.0352 found 309.0348.

[0173] A solution of freshly prepared lithium diisopropylamide (10.4 mL of a 0.31M stock solution, 3.22 mmol) cooled to -78°C was treated with 3,4-dichlorophenylacetic acid methyl ester (prepared as in Example 1, 642 mg 2.93 mmol) in tetrahydrofuran/1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (7.33 mL, 3:1). The resulting solution was stirred at -78°C for 45 min. A solution of 2-(2-iodomethyl-cyclopentyloxy)-tetrahydropyran (1.0 g, 3.22 mmol) in 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (1 mL) was then added. The reaction mixture was stirred at -78°C for 2 h. The reaction was then warmed to 25°C and was stirred at 25°C for 16 h. The reaction mixture was then quenched by the dropwise addition of a saturated aqueous ammonium chloride solution (10 mL). This mixture was poured into water (100 mL) and extracted with methylene chloride (3 x 50 mL). The organics were dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 90/10 hexanes/ethyl acetate) afforded 2-(3,4-dichloro-phenyl)-3-[2-(tetrahydro-pyran-2-yloxy)-cyclopentyl]-propionic acid methyl ester (880 mg, 74.8%) as a pale yellow oil: EI-HRMS m/e calcd for $C_{20}H_{26}Cl_2O_4$ (M^+) 400.1208 found 400.1203.

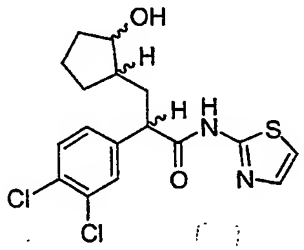
[0174] 2-(3,4-Dichloro-phenyl)-3-[2-(tetrahydro-pyran-2-yloxy)-cyclopentyl]-propionic acid methyl ester (400 mg, 0.99 mmol) and methylurea (110.7 mg, 1.49 mmol) in a solution of magnesium methoxide in methanol (7.4 wt.%, 2.85 mL, 1.99 mmol) was heated under reflux at 110°C for 6 h. The reaction mixture was then concentrated *in vacuo* and filtered through a plug of celite (ethyl acetate as eluent). Flash chromatography (Merck Silica gel 60, 230-400 mesh, 100% ethyl acetate) afforded 1-{2-(3,4-dichloro-phenyl)-3-[2-(tetrahydro-pyran-2-yloxy)-cyclopentyl]-propionyl}-3-methyl-urea (52 mg, 11.8%) as a white solid: mp 68-

70°C; FAB-HRMS m/e calcd for $C_{21}H_{28}Cl_2N_2O_4$ $(M+H)^+$ 443.1504, found 443.1499.

[0175] A solution of 1-{2-(3,4-dichloro-phenyl)-3-[2-(tetrahydro-pyran-2-yloxy)-cyclopentyl]-propionyl}-3-methyl-urea (45 mg, 0.10 mmol) in ethanol (1.01 mL) was treated with pyridinium *p*-toluenesulfonate (2.5 mg, 0.01 mmol). The reaction mixture was heated to 55°C for 5 h. At this time, the reaction was concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 100% ethyl acetate) afforded the 1-[2-(3,4-dichloro-phenyl)-3-(2-hydroxy-cyclopentyl)-propionyl]-3-methyl-urea (30.4 mg, 83.4%) as a white solid: mp 62-64°C; FAB-HRMS m/e calcd for $C_{16}H_{20}Cl_2N_2O_3$ $(M+H)^+$ 359.0929, found 359.0929.

Example 25

2-(3,4-Dichloro-phenyl)-3-(2-hydroxy-cyclopentyl)-N-thiazol-2-yl-propionamide

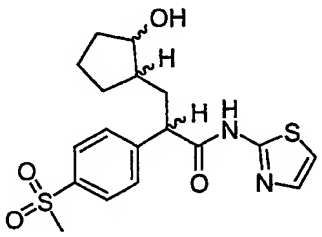


[0176] A solution of 2-(3,4-dichloro-phenyl)-3-[2-(tetrahydro-pyran-2-yloxy)-cyclopentyl]-propionic acid methyl ester (prepared as in Example 24, 2.3 g, 5.73 mmol) in ethanol (57.3 mL) at 25°C was treated with pyridinium *p*-toluenesulfonate (144 mg, 0.57 mmol). The resulting solution was heated under reflux for 18 h. At this time, the reaction was cooled to 25°C and concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 50/50 hexanes/ethyl acetate) afforded 2-(3,4-dichloro-phenyl)-3-(2-hydroxy-cyclopentyl)-propionic acid methyl ester (1.84 g, 100%) as a clear oil: EI-HRMS m/e calcd for $C_{15}H_{18}Cl_2O_3$ $(M+Na)^+$ 339.0527, found 339.0528.

[0177] A mixture of 2-(3,4-dichloro-phenyl)-3-(2-hydroxy-cyclopentyl)-propionic acid methyl ester (262.2 mg, 0.82 mmol) and 2-aminothiazole (165 mg, 1.65

mmol) in a solution of magnesium methoxide in methanol (7.4 wt.%, 2.60 mL, 1.81 mmol) was heated to 100°C for 18 h. At this time, the reaction mixture was cooled to 25°C and filtered through a pad of celite (ethyl acetate as eluent). The filtrate was then concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 75/25 ethyl acetate/hexanes) afforded the 2-(3,4-dichloro-phenyl)-3-(2-hydroxy-cyclopentyl)-N-thiazol-2-yl-propionamide (174 mg, 54.6%) as a white solid: mp 86-88°C; EI-HRMS m/e calcd for $C_{17}H_{18}Cl_2N_2O_2S$ $(M+Na)^+$ 407.0358, found 407.0361.

Example 26
3-(2-Hydroxy-cyclopentyl)-2-(4-methanesulfonyl-phenyl)-N-thiazol-2-yl-propionamide



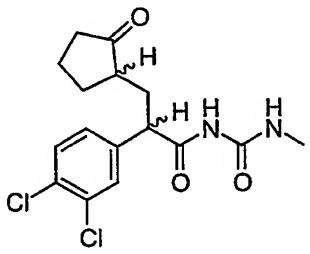
[0178] A solution of diisopropylamine (846 μL, 6.04 mmol) in dry tetrahydrofuran (4.4 mL) and 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (1.4 mL) was cooled to -78°C and then treated with a 2.5M solution of *n*-butyllithium in hexanes (2.4 mL, 6.04 mmol). The reaction mixture was stirred at -78°C for 30 min and then was treated with a solution of (4-methanesulfonyl-phenyl)-acetic acid methyl ester (prepared as in Example 8, 1.06 g, 4.64 mmol) in dry tetrahydrofuran (4.4 mL) and 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (1.4 mL). The resulting reaction mixture was allowed to stir at -78°C for 45 min and then the reaction mixture was treated with a solution of 2-(2-iodomethyl-cyclopentyloxy)-tetrahydropyran (prepared as in Example 24, 1.87 g, 6.04 mmol) in a small amount of dry tetrahydrofuran. The reaction mixture was stirred at -78°C and then allowed to warm to 25°C, where it was stirred for 68 h. The reaction mixture was quenched with water (100 mL) and then concentrated *in vacuo* to remove tetrahydrofuran. The aqueous residue was extracted with ethyl acetate (2 x 100 mL). The combined organic extracts were dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 70-230 mesh,

7/3 hexanes/ethyl acetate) afforded the 2-(4-methanesulfonyl-phenyl)-3-[2-(tetrahydropyran-2-yloxy)-cyclopentyl]-propionic acid methyl ester (859.2 mg, 45%) as a light yellow oil: FAB-HRMS m/e calcd for $C_{21}H_{30}O_6S$ $(M+H)^+$ 411.1841, found 411.1831.

[0179] 2-Aminothiazole (314.3 mg, 3.14 mmol) and 2-(4-methanesulfonyl-phenyl)-3-[2-(tetrahydropyran-2-yloxy)-cyclopentyl]-propionic acid methyl ester (859.2 g, 2.09 mmol) were treated with a solution of magnesium methoxide in methanol (7.4 wt.%, 12 mL, 8.37 mmol). The resulting reaction mixture was heated under reflux for 24 h. The reaction mixture was allowed to cool to 25°C and then filtered through a pad of celite. The pad of celite was washed well with ethyl acetate until the washings indicated the absence of product by thin layer chromatography. The filtrate was then washed with a 10% aqueous hydrochloric acid solution (3 x 100 mL). The organic layer was then dried over magnesium sulfate, filtered, and concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 70-230 mesh, 1/1 hexanes/ethyl acetate) afforded 2-(4-methanesulfonyl-phenyl)-3-[2-(tetrahydropyran-2-yloxy)-cyclopentyl]-N-thiazol-2-yl-propionamide (377.4 mg, 38%) as a yellow oil: EI-HRMS m/e calcd for $C_{23}H_{30}N_2O_5S_2$ (M^+) 478.1596, found 478.1604.

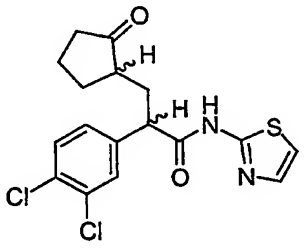
[0180] A solution of 2-(4-methanesulfonyl-phenyl)-3-[2-(tetrahydropyran-2-yloxy)-cyclopentyl]-N-thiazol-2-yl-propionamide (350.8 mg, 0.73 mmol) in ethanol (7.3 mL) was treated with pyridinium *p*-toluenesulfonate (18.4 mg, 0.073 mmol). The resulting reaction mixture was heated at 60°C for 4 h. The reaction mixture was allowed to cool to 25°C and then concentrated *in vacuo*. The resulting yellow residue was diluted with ethyl acetate (100 mL) and then washed with a saturated aqueous sodium chloride solution (1 x 100 mL). The organic layer was dried over magnesium sulfate, filtered, and concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 70-230 mesh, 1/3 hexanes/ethyl acetate) afforded the 3-(2-hydroxy-cyclopentyl)-2-(4-methanesulfonyl-phenyl)-N-thiazol-2-yl-propionamide (77.0 mg, 27%) as a white solid: mp 205-206°C; EI-HRMS m/e calcd for $C_{18}H_{22}N_2O_4S_2$ (M^+) 394.1021, found 394.1018.

Example 27
1-[2-(3,4-Dichloro-phenyl)-3-(2-oxo-cyclopentyl)-propionyl]-3-methyl-urea



[0181] A solution of 1-[2-(3,4-dichloro-phenyl)-3-(2-hydroxy-cyclopentyl)-propionyl]-3-methyl-urea (prepared as in Example 24, 28.1 mg, 0.07 mmol) in methylene chloride (0.78 mL) was treated with pyridinium chlorochromate (20 wt.% on basic alumina, 101 mg, 0.09 mmol). The reaction mixture was stirred at 25°C for 4 h. At this time, the reaction was filtered through a plug of celite (ethyl acetate as eluent). The filtrate was concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 100% ethyl acetate) afforded 1-[2-(3,4-dchloro-phenyl)-3-(2-oxo-cyclopentyl)-propionyl]-3-methyl-urea (21.9 mg, 78.4%) as a white foam: mp 63-65°C; FAB-HRMS m/e calcd for $C_{16}H_{18}Cl_2N_2O_3$ $(M+H)^+$ 357.0773, found 357.0780.

Example 28
2-(3,4-Dichloro-phenyl)-3-(2-oxo-cyclopentyl)-N-thiazol-2-yl-propionamide

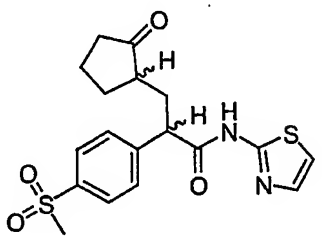


[0182] A solution of 2-(3,4-dichloro-phenyl)-3-(2-hydroxy-cyclopentyl)-N-thiazol-2-yl-propionamide (prepared as in Example 25, 162.5 mg, 0.42 mmol) in methylene chloride (4.2 mL) was treated with pyridinium chlorochromate (20 wt.% on basic alumina, 545 mg, 0.50 mmol). The reaction mixture was stirred at 25°C for 2h. At this time, the reaction was filtered through a plug of celite (ethyl acetate as eluent). The filtrate was concentrated *in vacuo*. Flash chromatography (Merck

Silica gel 60, 230-400 mesh, 50/50 hexanes/ethyl acetate) afforded 2-(3,4-dichloro-phenyl)-3-(2-oxo-cyclopentyl)-N-thiazol-2-yl-propionamide (64.8 mg, 40.1%) as a light tan solid: mp 79-81°C; EI-HRMS m/e calcd for $C_{17}H_{16}Cl_2N_2O_2S$ $(M+H)^+$ 383.0383, found 383.0384.

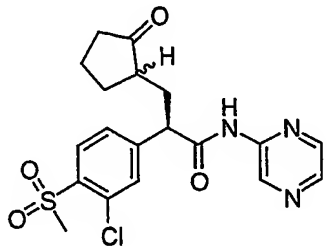
Example 29

2-(4-Methanesulfonyl-phenyl)-3-(2-oxo-cyclopentyl)-N-thiazol-2-yl-propionamide



[0183] A solution of 3-(2-hydroxy-cyclopentyl)-2-(4-methanesulfonyl-phenyl)-N-thiazol-2-yl-propionamide (prepared as in Example 26, 72.4 mg, 0.184 mmol) in methylene chloride (1.8 mL) was treated with pyridinium chlorochromate (20 wt.% on basic alumina, 237.3 mg, 0.22 mmol). The resulting reaction mixture was stirred at 25°C for 3 h, at which time, thin layer chromatography indicated a small amount of the 3-(2-hydroxy-cyclopentyl)-2-(4-methanesulfonyl-phenyl)-N-thiazol-2-yl-propionamide. The reaction mixture was then treated with an additional amount of pyridinium chlorochromate (20 wt.% on basic alumina, 237.3 mg, 0.220 mmol). The reaction mixture was allowed to stir at 25°C for 4 h and then filtered through a pad of celite. The pad of celite was washed well with ethyl acetate until the washings indicated the absence of product by thin layer chromatography. The filtrate was then concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 3/7 hexanes/ethyl acetate) afforded 2-(4-methanesulfonyl-phenyl)-3-(2-oxo-cyclopentyl)-N-thiazol-2-yl-propionamide (10.2 mg, 14%) as a white solid: mp 228-230°C; EI-HRMS m/e calcd for $C_{18}H_{20}N_2O_4S_2$ (M^+) 392.0865, found 392.0871.

Example 30
2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-3-(2-oxo-cyclopentyl)-N-pyrazin-2-yl-propionamide



[0184] A mixture of (3-chloro-4-methylsulfanyl-phenyl)-acetic acid (prepared as in Example 4, 10.48 g, 48.4 mmol) and potassium carbonate (20.1 g, 145.1 mmol) in acetone (65 mL) was cooled to -10°C. The pale yellow slurry was then treated dropwise with trimethylacetyl chloride (6.25 mL, 50.8 mmol) while maintaining the temperature below -10°C. The resulting reaction mixture was stirred at -10°C for 15 min and then allowed to warm to 0°C where it was stirred for 10 min. The reaction mixture was re-cooled to -10°C and then treated with (1R, 2R)-(-)-pseudoephedrine (11.99 g, 72.5 mmol), resulting in an exotherm. The reaction mixture was stirred -10°C for 10 min and then warmed to 25°C, where it was stirred for 1 h. After such time, thin layer chromatography analysis indicated that the reaction was complete. The reaction mixture was then quenched with water (50 mL) and then extracted with ethyl acetate (1 x 100 mL). The organic layer was washed with water (2 x 40 mL). The aqueous layers were combined and back-extracted with ethyl acetate (2 x 50 mL). The combined organic layers were dried over magnesium sulfate, filtered, and concentrated *in vacuo*. The crude material was recrystallized from ethyl acetate (45 mL) and hexanes (80 mL) to afford 2-(3-chloro-4-methylsulfanyl-phenyl)-N-[2(R)-hydroxy-1(R)-methyl-2(R)-phenyl-ethyl]-N-methyl-acetamide (13.75 g, 78%) as a light yellow solid: mp 111.5-112.9°C; $[\alpha]^{23}_{589}$ = –97.2° (c=0.104, chloroform); FAB-HRMS m/e calcd for $C_{19}H_{22}ClNSO_2$ $(M+H)^+$ 364.1138, found 364.1142.

[0185] A solution of 1,1,1,3,3,3-hexamethyldisilizane (14.5 mL, 68.7 mmol) in tetrahydrofuran (45.8 mL) cooled to -45°C was treated with a 2.5M solution of *n*-butyllithium in hexanes (25.8 mL, 63.2 mmol). The resulting solution was stirred

at -45°C for 30 min. At this time, the reaction was treated with a solution of 2-(3-chloro-4-methylsulfanyl-phenyl)-N-[2(R)-hydroxy-1(R)-methyl-2(R)-phenyl-ethyl]-N-methyl-acetamide (10.0 g, 27.48 mmol) in tetrahydrofuran (45.86 mL). Upon complete addition, the reaction was warmed to 0°C and was stirred at 0°C for 30 min. At this time, the reaction was re-cooled to -45°C and then was treated with 2-(2-iodomethyl-cyclopentyloxy)-tetrahydropyran (prepared as in Example 24, 12.8 g, 41.22 mmol) in 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (8.4 mL). At this time, the reaction was warmed to 0°C, where it was stirred for 3 h. At this time, the reaction was diluted with a saturated aqueous sodium chloride solution (100 mL). The phases were partitioned. The aqueous phase was extracted into ethyl acetate (3 x 50 mL). The combined organics were washed with a 10% aqueous hydrochloric acid solution and an aqueous sodium bicarbonate solution, dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 75/25 ethyl acetate/hexanes) afforded 2(R)-(3-chloro-4-methylsulfanyl-phenyl)-N-(2(R)-hydroxy-1(R)-methyl-2(R)-phenyl-ethyl)-N-methyl-3-[2-(tetrahydro-pyran-2-yloxy)-cyclopentyl]-propionamide (10.6 g, 70.6%) as a yellow foam: EI-HRMS m/e calcd for $C_{30}H_{40}ClNO_4S$ $(M+Na)^+$ 568.2259, found 568.2262.

[0186] A solution of 2(R)-(3-chloro-4-methylsulfanyl-phenyl)-N-(2(R)-hydroxy-1(R)-methyl-2(R)-phenyl-ethyl)-N-methyl-3-[2-(tetrahydro-pyran-2-yloxy)-cyclopentyl]-propionamide (1.04 g, 1.91 mmol) in ethanol (19.1 mL) was treated with pyridinium *p*-toluenesulfonate (48 mg, 0.19 mmol). The resulting solution was heated to 55°C for 2 h. At this time, the reaction was concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 75/25 ethyl acetate/hexanes) afforded 2(R)-(3-chloro-4-methylsulfanyl-phenyl)-3-(2-hydroxy-cyclopentyl)-N-(2(R)-hydroxy-1(R)-methyl-2(R)-phenyl-ethyl)-N-methyl-propionamide (0.82 g, 93.0%) as a white foam: EI-HRMS m/e calcd for $C_{25}H_{32}ClNO_3S$ $(M+Na)^+$ 484.1684, found 484.1674.

[0187] A mixture of 2(R)-(3-chloro-4-methylsulfanyl-phenyl)-3-(2-hydroxy-cyclopentyl)-N-(2(R)-hydroxy-1(R)-methyl-2(R)-phenyl-ethyl)-N-methyl-propionamide (3.63 g, 7.85 mmol), N-methylmorpholine N-oxide (2.76 g, 23.5

mmol), and powdered molecular sieves (7.85 g) in methylene chloride (15.7 mL) at 25°C was treated with tetrapropylammonium perrhuthenate (276 mg, 0.78 mmol). The resulting mixture was stirred at 25°C for 20 min. At this time, the reaction was filtered through a pad of silica (ethyl acetate as eluent). The filtrate was concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 75/25 ethyl acetate/hexanes) afforded 2(R)-(3-chloro-4-methylsulfanyl-phenyl)-N-methyl-N-(1(R)-methyl-2-oxo-2(R)-phenyl-ethyl)-3-(2-oxo-cyclopentyl)-propionamide (2.75 g, 76.5%) as a white foam: EI-HRMS m/e calcd for $C_{25}H_{28}ClNO_3S(M^+)$ 457.1478, found 457.1489.

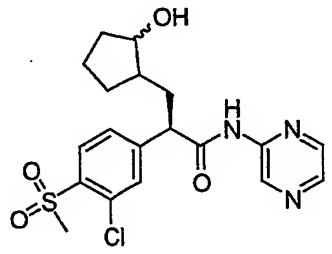
[0188] A solution of 2(R)-(3-chloro-4-methylsulfanyl-phenyl)-N-methyl-N-(1(R)-methyl-2-oxo-2(R)-phenyl-ethyl)-3-(2-oxo-cyclopentyl)-propionamide (2.75 g, 6.0 mmol) in dioxane (9.38 mL) was treated with an 18M aqueous hydrochloric acid solution (9.38 mL). The reaction was then heated to 120°C for 18 h. At this time, the reaction was cooled to 25°C, diluted with water (100 mL), and extracted with a 90/10 methylene chloride/methanol solution (3 x 100 mL). The organics were dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, ethyl acetate) afforded 2(R)-(3-chloro-4-methylsulfanyl-phenyl)-3-(2-oxo-cyclopentyl)-propionic acid (1.54 g, 82%) as a yellow oil: EI-HRMS m/e calcd for $C_{15}H_{17}ClO_3S$ (M^+) 312.0587, found 312.0581.

[0189] A solution of 2(R)-(3-chloro-4-methylsulfanyl-phenyl)-3-(2-oxo-cyclopentyl)-propionic acid (683.2 mg, 2.18 mmol), formic acid (2.47 mL, 65.5 mmol), and water (0.41 mL) cooled to 0°C was treated with a 30% aqueous hydrogen peroxide solution (1.11 mL, 10.9 mmol). The reaction was stirred at 0°C for 1 h. At this time, the reaction was treated with a saturated aqueous sodium sulfite solution. The resulting solution was poured into water (50 mL) and was then extracted into methylene chloride (3 x 50 mL). The organics were dried over sodium sulfate, filtered, and concentrated *in vacuo* to afford 2(R)-(3-chloro-4-methanesulfinyl-phenyl)-3-(2-oxo-cyclopentyl)-propionic acid (724 mg, 100%) as a white foam: EI-HRMS m/e calcd for $C_{15}H_{17}ClO_4S$ $(M+Na)^+$ 351.0428, found 351.0433.

[0190] A solution of potassium permanganate (101 mg, 0.64 mmol) in water (1.83 mL) was treated with a solution of 2(R)-(3-chloro-4-methanesulfinyl-phenyl)-3-(2-oxo-cyclopentyl)-propionic acid (191.5 mg, 0.58 mmol) in methanol (5.8 mL). The reaction was stirred at 25°C for 1 h. At this time, the reaction was diluted with methanol and then was filtered through a pad of celite. The filtrate was concentrated *in vacuo* and was azeotroped with acetonitrile. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 90/10 methylene chloride/methanol with glacial acetic acid) afforded 2(R)-(3-chloro-4-methanesulfonyl-phenyl)-3-(2-oxo-cyclopentyl)-propionic acid (87 mg, 43%) as an off-white foam: EI-HRMS m/e calcd for $C_{15}H_{17}ClO_5S$ $(M-H_2O)^+$ 326.0379, found 326.0378.

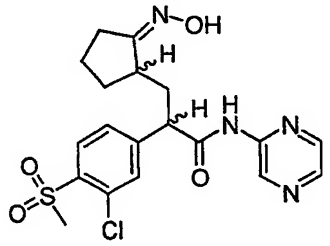
[0191] A solution of 2(R)-(3-chloro-4-methanesulfonyl-phenyl)-3-(2-oxo-cyclopentyl)-propionic acid (125 mg, 0.36 mmol) in methylene chloride (3.62 mL) cooled to 0°C was treated with a 2.0M solution of oxalyl chloride in methylene chloride (0.20 mL, 0.39 mmol) and a few drops of *N,N*-dimethylformamide. The reaction mixture was stirred at 0°C for 10 min and at 25°C for 20 min. The reaction mixture was then treated with a solution of 2-aminopyrazine (76 mg, 0.80 mmol) and pyridine (0.06 mL, 0.80 mmol) in tetrahydrofuran (1.81 mL). This solution was stirred at 25°C for 1 h. At this time, the reaction was concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 75/25 ethyl acetate/hexanes) afforded 2(R)-(3-chloro-4-methanesulfonyl-phenyl)-3-(2-oxo-cyclopentyl)-N-pyrazin-2-yl-propionamide (77.6 mg, 50.7%) as a white foam: EI-HRMS m/e calcd for $C_{19}H_{20}ClN_3O_4S$ (M^+) 421.0863, found 421.0868.

Example 31
2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-3-(2-hydroxy-cyclopentyl)-N-pyrazin-2-yl-propionamide



[0192] A solution of 2(R)-(3-chloro-4-methanesulfonyl-phenyl)-3-(2-oxo-cyclopentyl)-N-pyrazin-2-yl-propionamide (prepared as in Example 30, 13.5 mg, 0.03 mmol) in ethanol (0.32 mL) cooled to 0°C was treated with sodium borohydride (1.2 mg, 0.03 mmol). The reaction mixture was stirred at 0°C for 20 min. At this time, the reaction was diluted with water (25 mL). The layers were separated. The aqueous layer was extracted with ethyl acetate (3 x 30 mL). The combined organics were dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, ethyl acetate) afforded 2(R)-(3-chloro-4-methanesulfonyl-phenyl)-3-(2-hydroxy-cyclopentyl)-N-pyrazin-2-yl-propionamide (10.1 mg, 75.5%) as a white foam: EIHRMS m/e calcd for $C_{19}H_{22}ClN_3O_4S$ $(M+Na)^+$ 446.0912, found 446.0916.

Example 32
2-(3-Chloro-4-methanesulfonyl-phenyl)-3-(2-hydroxyimino-cyclopentyl)-N-pyrazin-2-yl-propionamide



[0193] A mixture of 2(R)-(3-chloro-4-methylsulfanyl-phenyl)-3-(2-hydroxy-cyclopentyl)-N-(2(R)-hydroxy-1(R)-methyl-2(R)-phenyl-ethyl)-N-methyl-propionamide (prepared as in Example 30, 3.63 g, 7.85 mmol), N-methylmorpholine N-oxide (7.37 g, 15.95 mmol), and powdered molecular sieves

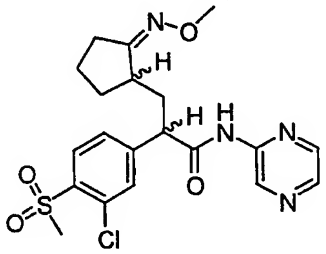
(32 g) in methylene chloride (63.8 mL) at 0°C was treated with tetrapropylammonium perrhuthenate (1.12 mg, 3.19 mmol). The resulting mixture was stirred at 0°C for 1 h and at 25°C for 3 h. At this time, the reaction was filtered through a pad of silica (ethyl acetate as eluent). The filtrate was concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 75/25 ethyl acetate/hexanes) afforded 2(R)-(3-chloro-4-methylsulfanyl-phenyl)-N-methyl-N-(1(R)-methyl-2-oxo-2(R)-phenyl-ethyl)-3-(2-oxo-cyclopentyl)-propionamide (5.03 g, 68.8%) and 2(R)-(3-chloro-4-methylsulfonyl-phenyl)-N-methyl-N-(1(R)-methyl-2-oxo-2(R)-phenyl-ethyl)-3-(2-oxo-cyclopentyl)-propionamide (1.29 g, 16.5%) as a white foam: EI-HRMS m/e calcd for $C_{25}H_{28}ClNO_5S$ $(M+Na)^+$ 512.1269, found 512.1273.

[0194] A solution of 2(R)-(3-chloro-4-methylsulfonyl-phenyl)-N-methyl-N-(1(R)-methyl-2-oxo-2(R)-phenyl-ethyl)-3-(2-oxo-cyclopentyl)-propionamide (1.29 g, 2.63 mmol) in dioxane (4.43 mL) was treated with an 18M aqueous hydrochloric acid solution (4.43 mL). The reaction was then heated to 120°C for 18 h. At this time, the reaction was cooled to 25°C, diluted with water (50 mL), and extracted with a 90/10 methylene chloride/methanol solution (3 x 100 mL). The organics were dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, ethyl acetate) afforded racemic 2-(3-chloro-4-methylsulfonyl-phenyl)-3-(2-oxo-cyclopentyl)-propionic acid (563.3 mg, 62.1%) as a light tan foam. A solution of 2-(3-chloro-4-methanesulfonyl-phenyl)-3-(2-oxo-cyclopentyl)-propionic acid (563.3 mg, 1.6 mmol) in methylene chloride (16.4 mL) cooled to 0°C was treated with a 2.0M solution of oxalyl chloride in methylene chloride (0.90 mL, 1.80 mmol) and a few drops of *N,N*-dimethylformamide. The reaction mixture was stirred at 0°C for 10 min and at 25°C for 20 min. The reaction mixture was then treated with a solution of 2-aminopyrazine (342 mg, 3.60 mmol) and pyridine (0.3 mL, 3.60 mmol) in tetrahydrofuran (8.2 mL). This solution was stirred at 25°C for 18 h. At this time, the reaction was concentrated *in vacuo*. The residue was dissolved in methylene chloride (100 mL) and washed with a 1N aqueous hydrochloric acid solution (2 x 100 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 75/25 ethyl acetate/hexanes)

afforded 2-(3-chloro-4-methanesulfonyl-phenyl)-3-(2-oxo-cyclopentyl)-N-pyrazin-2-yl-propionamide (198.8 mg, 28.9%) as a clear oil.

[0195] A solution of 2-(3-chloro-4-methanesulfonyl-phenyl)-3-(2-oxo-cyclopentyl)-N-pyrazin-2-yl-propionamide (99.3 mg, 0.23 mmol) in pyridine (0.69 mL) and methanol (0.69 mL) at 25°C was treated with hydroxylamine hydrochloride (24.5 mg, 0.35 mmol). The reaction mixture was heated under reflux for 12 h. At this time, the reaction was cooled to 25°C and was concentrated *in vacuo*. The resulting residue was dissolved in ethyl acetate (75 mL) and was washed with a 1N aqueous sodium hydroxide solution (1 x 50 mL) and a saturated aqueous sodium chloride solution (1 x 50 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 75/25 ethyl acetate/hexanes) afforded 2-(3-chloro-4-methanesulfonyl-phenyl)-3-(2-hydroxyimino-cyclopentyl)-N-pyrazin-2-yl-propionamide (5.6 mg, 5.4%) as a white wax: (ES)$^-$-HRMS m/e calcd for $C_{19}H_{21}ClN_4O_4S$ $(M-H)^-$ 435.0899, found 435.0902.

Example 33
2-(3-Chloro-4-methanesulfonyl-phenyl)-3-(2-methoxyimino-cyclopentyl)-N-pyrazin-2-yl-propionamide

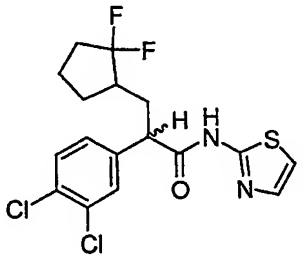


[0196] A solution of 2-(3-chloro-4-methanesulfonyl-phenyl)-3-(2-oxo-cyclopentyl)-N-pyrazin-2-yl-propionamide (prepared as in Example 32, 99.5 mg, 0.23 mmol) in pyridine (0.69 mL) and methanol (0.69 mL) at 25°C was treated with methoxyamine hydrochloride (30 mg, 0.35 mmol). The reaction mixture was heated under reflux for 12 h. At this time, the reaction was cooled to 25°C and was concentrated *in vacuo*. The resulting residue was dissolved in ethyl acetate (75 mL) and was washed with a 1N aqueous sodium hydroxide solution (1 x 50

mL) and a saturated aqueous sodium chloride solution (1 x 50 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 75/25 ethyl acetate/hexanes) afforded 2-(3-chloro-4-methanesulfonyl-phenyl)-3-(2-methoxyimino-cyclopentyl)-N-pyrazin-2-yl-propionamide (92.7 mg, 87.2%) as a white wax: mp 74-76°C; FAB-HRMS m/e calcd for $C_{20}H_{23}ClN_4O_4S$ $(M+Na)^+$ 473.1021, found 473.1024.

Example 34

2-(3,4-Dichloro-phenyl)-3-(2,2-difluoro-cyclopentyl)-N-thiazol-2-yl-propionamide



[0197] A mixture of 2-(3,4-dichloro-phenyl)-3-(2-hydroxy-cyclopentyl)-propionic acid methyl ester (prepared as in Example 25, 408.5 mg, 1.28 mmol), *N*-methylmorpholine *N*-oxide (679 mg, 5.79 mmol), and powdered molecular sieves (1.29 g) in methylene chloride (2.6 mL) at 25°C was treated with tetrapropylammonium perrhuthenate (45 mg, 0.12 mmol). The resulting mixture was stirred at 25°C for 2 h. At this time, the reaction was filtered through a pad of celite (ethyl acetate as eluent). The filtrate was concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 75/25 hexanes/ethyl acetate) afforded 2-(3,4-dichloro-phenyl)-3-(2-oxo-cyclopentyl)-propionic acid methyl ester (278.2 mg, 68.5%) as a clear oil: EI-HRMS m/e calcd for $C_{15}H_{16}Cl_2O_3$ (M^+) 314.0476, found 314.0476.

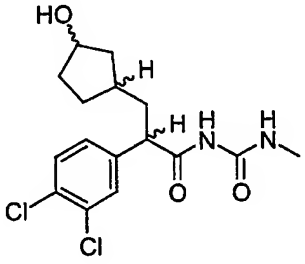
[0198] A solution of 2-(3,4-dichloro-phenyl)-3-(2-oxo-cyclopentyl)-propionic acid methyl ester (270 mg, 0.85 mmol) in methylene chloride (0.43 mL) was treated with diethylaminosulfur trifluoride (0.17 mL, 1.28 mmol). The resulting mixture was heated at 60°C for 18 h. At this time, the reaction was cooled to 25°C and was diluted with water (50 mL). This solution was extracted into methylene chloride (3 x 30 mL). The organics were dried over sodium sulfate, filtered, and

concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 75/25 hexanes/ethyl acetate) afforded 2-(3,4-dichloro-phenyl)-3-(2,2-difluoro-cyclopentyl)-propionic acid methyl ester (278.2 mg, 68.5%) as a light tan oil: EI-HRMS m/e calcd for $C_{15}H_{16}Cl_2F_2O_2$ (M^+) 336.0495, found 336.0510.

[0199] A mixture of 2-(3,4-dichloro-phenyl)-3-(2,2-difluoro-cyclopentyl)-propionic acid methyl ester (265 mg, 0.73 mmol) and 2-aminothiazole (18 mg, 0.18 mmol) in a solution of magnesium methoxide in methanol (7.4 wt.%, 0.28 mL, 0.19 mmol) was heated to 110°C for 18 h. At this time, the reaction mixture was cooled to 25°C and then concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 50/50 hexanes/ethyl acetate) afforded 2-(3,4-dichloro-phenyl)-3-(2,2-difluoro-cyclopentyl)-N-thiazol-2-yl-propionamide (8.3 mg, 23%) as a yellow oil: EI-HRMS m/e calcd for $C_{17}H_{16}F_2Cl_2N_2OS$ $(M+H)^+$ 405.0402, found 405.0407.

Example 35

1-[2-(3,4-Dichloro-phenyl)-3-(3-hydroxy-cyclopentyl)-propionyl]-3-methyl-urea



[0200] A solution of 3-iodomethyl-cyclopentanone (12.84 g, 57.31 mmol, prepared according to *J. Org. Chem.* **1981**, *46*, 2412-2414) in methanol (143 mL) was cooled to 0°C and then slowly treated with sodium borohydride powder (2.38 g, 63.04 mmol). The resulting reaction mixture was stirred at 0°C for 40 min and then slowly quenched with water (100 mL). The reaction mixture was then concentrated *in vacuo* to remove methanol. The resulting aqueous residue was extracted with diethyl ether (3 x 100 mL). The combined organic extracts were dried over magnesium sulfate, filtered, and concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 70-230 mesh, 100% methylene chloride)

afforded 3-iodomethyl-cyclopentanol (7.71 g, 59%) as a green liquid: EI-HRMS m/e calcd for $C_6H_{11}IO$ (M^+) 225.9855, found 225.9856.

[0201] A solution of 3-iodomethyl-cyclopentanol (7.71 g, 34.10 mmol) in methylene chloride (171 mL) was treated with 3,4-dihydro-2*H*-pyran (4.7 ml, 51.16 mmol) and pyridinium *p*-toluenesulfonate (857.1 mg, 3.41 mmol). The resulting reaction mixture was stirred at 25°C for 24 h. The reaction mixture was then washed with a saturated aqueous sodium chloride solution (1 x 200 mL). The organic layer was dried over magnesium sulfate, filtered, and concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 70-230 mesh, 19/1 petroleum ether/diethyl ether) afforded 2-(3-iodomethyl-cyclopentyloxy)-tetrahydropyran (7.91 g, 75%) as a yellow oil: EI-HRMS m/e calcd for $C_{11}H_{19}IO_2$ (M^+) 310.0430, found 310.0433.

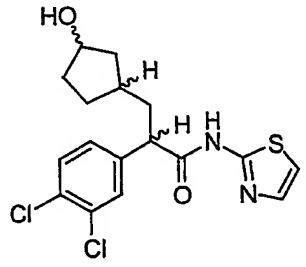
[0202] A solution of freshly prepared lithium diisopropylamide (23 mL of a 0.31M stock solution, 7.13 mmol) cooled to -78°C was treated with (3,4-dichloro-phenyl)-acetic acid methyl ester (prepared as in Example 1, 1.42 g, 6.48 mmol) in tetrahydrofuran/1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (16.15 mL, 3:1). The resulting solution was stirred at -78°C for 45 min. A solution of 2-(3-iodomethyl-cyclopentyloxy)-tetrahydropyran (2.21 g, 7.13 mmol) in 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (1 mL) was then added. The reaction mixture was stirred at -78°C for 2 h. The reaction was then warmed to 25°C and was stirred at 25°C for 18 h. The reaction mixture was then quenched by the dropwise addition of a saturated aqueous ammonium chloride solution (10 mL). This mixture was poured into water (100 mL) and extracted with methylene chloride (3 x 50 mL). The organics were washed with a saturated aqueous lithium chloride solution (1 x 100 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 70/30 hexanes/ethyl acetate) afforded 2-(3,4-dichloro-phenyl)-3-[3-(tetrahydro-pyran-2-yloxy)-cyclopentyl]-propionic acid methyl ester (2.12 g, 81.6%) as a clear oil: EI-HRMS m/e calcd for $C_{20}H_{26}Cl_2O_4$ $(M+Na)^+$ 423.1106 found 423.1093.

[0203] 2-(3,4-Dichloro-phenyl)-3-[3-(tetrahydro-pyran-2-yloxy)-cyclopentyl]-propionic acid methyl ester (1.60 mg, 3.98 mmol) and methylurea (443 mg, 5.98 mmol) in a solution of magnesium methoxide in methanol (7.4 wt.%, 11.4 mL, 7.97 mmol) was heated under reflux at 100°C for 18 h. The reaction mixture was then concentrated *in vacuo* and filtered through a plug of celite (100% ethyl acetate as eluent). Flash chromatography (Merck Silica gel 60, 230-400 mesh, 50/50 hexanes/ethyl acetate) afforded 1-{2-(3,4-dichloro-phenyl)-3-[3-(tetrahydro-pyran-2-yloxy)-cyclopentyl]-propionyl}-3-methyl-urea (160.6 mg, 9.1%) as a white solid: mp 62-65°C; FAB-HRMS m/e calcd for $C_{21}H_{28}Cl_2N_2O_4$ $(M+Na)^+$ 465.1324, found 465.1324.

[0204] A solution of 1-{2-(3,4-dichloro-phenyl)-3-[3-(tetrahydro-pyran-2-yloxy)-cyclopentyl]-propionyl}-3-methyl-urea (150.7 mg, 0.33 mmol) in ethanol (3.4 mL) was treated with pyridinium *p*-toluenesulfonate (8.54 mg, 0.03 mmol). The reaction mixture was heated to 60°C for 18 h. At this time, the reaction was concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 80/20 methylene chloride/methanol) afforded 1-[2-(3,4-dichloro-phenyl)-3-(3-hydroxy-cyclopentyl)-propionyl]-3-methyl-urea (102.2 mg, 83.7%) as a white solid: mp 82-84°C; FAB-HRMS m/e calcd for $C_{16}H_{20}Cl_2N_2O_3$ $(M+H)^+$ 359.0929, found 359.0936.

Example 36

2-(3,4-Dichloro-phenyl)-3-(3-hydroxy-cyclopentyl)-N-thiazol-2-yl-propionamide

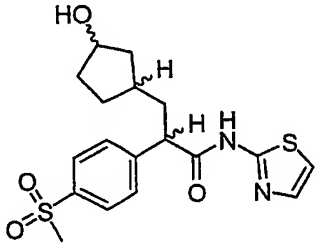


[0205] A solution of 2-(3,4-dichloro-phenyl)-3-[3-(tetrahydro-pyran-2-yloxy)-cyclopentyl]-propionic acid methyl ester (prepared as in Example 35, 1.03 g, 2.57 mmol) in methanol (6.4 mL) was treated with Amberlyst® 15 ion exchange resin (77 mg). The resulting reaction mixture was stirred at 25°C for 16 h and then was

heated at 45°C for 1 h. The reaction mixture was allowed to cool to 25°C, and the resin was then filtered off and then washed well with ethyl acetate. The filtrate was concentrated *in vacuo* to afford 2-(3,4-dichloro-phenyl)-3-(3-hydroxy-cyclopentyl)-propionic acid methyl ester (807.7 mg, 99%) as a yellow oil which was used without further purification.

[0206] A mixture of 2-(3,4-dichloro-phenyl)-3-(3-hydroxy-cyclopentyl)-propionic acid methyl ester (25 mg, 0.08 mmol) and 2-aminothiazole (9.4 mg, 0.09 mmol) in a solution of magnesium methoxide in methanol (7.4 wt.%, 0.22 mL, 0.15 mmol) was heated to 110°C for 18 h. At this time, the reaction mixture was cooled to 25°C and then filtered through a pad of celite (methylene chloride as eluent). The filtrate was washed with a 1N aqueous hydrochloric acid solution. The organics were dried over sodium sulfate, filtered and then concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 75/25 ethyl acetate/hexanes) afforded 2-(3,4-dichloro-phenyl)-3-(3-hydroxy-cyclopentyl)-N-thiazol-2-yl-propionamide (16.4 mg, 54%) as light tan oil: EI-HRMS m/e calcd for $C_{17}H_{18}Cl_2N_2O_2S$ $(M+H)^+$ 385.0537, found 385.0542.

Example 37
3-(3-Hydroxy-cyclopentyl)-2-(4-methanesulfonyl-phenyl)-N-thiazol-2-yl-propionamide



[0207] A solution of diisopropylamine (810 μL, 5.78 mmol) in dry tetrahydrofuran (4.5 mL) and 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (1.5 mL) cooled to -78°C was treated with a 2.5M solution of *n*-butyllithium in hexanes (2.3 mL, 5.78 mmol). The reaction mixture was stirred at -78°C for 30 min and then treated with a solution of (4-methanesulfonyl-phenyl)-acetic acid methyl ester (prepared as in Example 8, 1.10 g, 4.82 mmol) in dry tetrahydrofuran (4.5 mL) and 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (1.5 mL). The resulting

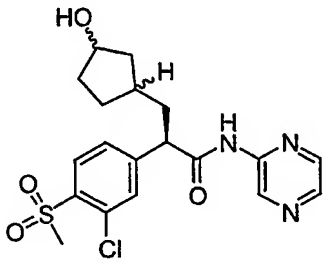
reaction mixture was allowed to stir at -78°C for 1 h and then the reaction mixture was treated with a solution of 2-(3-iodomethyl-cyclopentyloxy)-tetrahydropyran (prepared as in Example 35, 1.94 g, 6.26 mmol) in a small amount of dry tetrahydrofuran. The reaction mixture was stirred at -78°C for 10 min and then allowed to warm to 25°C, where it was stirred for 3 d. The reaction mixture was quenched with water (50 mL) and then concentrated *in vacuo* to remove tetrahydrofuran. The aqueous residue was further diluted with water (100 mL) and then extracted with ethyl acetate (3 x 100 mL). The combined organic extracts were dried over magnesium sulfate, filtered, and concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 70-230 mesh, 7/3 hexanes/ethyl acetate) afforded the 2-(4-methanesulfonyl-phenyl)-3-[3-(tetrahydropyran-2-yloxy)-cyclopentyl]-propionic acid methyl ester (1.07 g, 54%) as a yellow oil: FAB-HRMS m/e calcd for $C_{21}H_{30}O_6S$ $(M+H)^+$ 411.1841, found 411.1851.

[0208] 2-Aminothiazole (259 mg, 2.58 mmol) and 2-(4-methanesulfonyl-phenyl)-3-[3-(tetrahydropyran-2-yloxy)-cyclopentyl]-propionic acid methyl ester (1.06 g, 2.58 mmol) were treated with a solution of magnesium methoxide in methanol (7.4 wt.%, 14.76 mL, 10.32 mmol). The reaction mixture was then concentrated *in vacuo* to approximately one-half the volume of methanol. The resulting reaction mixture was heated under reflux for 24 h. The reaction mixture was allowed to cool to 25°C and then filtered through a pad of celite. The pad of celite was washed well with ethyl acetate until the washings indicated the absence of product by thin layer chromatography. The filtrate was then washed with a saturated aqueous sodium chloride solution, dried over magnesium sulfate, filtered, and concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 1/2 hexanes/ethyl acetate) afforded 2-(4-methanesulfonyl-phenyl)-3-[3-(tetrahydropyran-2-yloxy)-cyclopentyl]-N-thiazol-2-yl-propionamide (494 mg, 40%) as a yellow foam: mp 68-70°C (foam to gel); FAB-HRMS m/e calcd for $C_{23}H_{30}N_2O_5S_2$ $(M+H)^+$ 479.1674, found 479.1666.

[0209] A solution of 2-(4-methanesulfonyl-phenyl)-3-[3-(tetrahydropyran-2-yloxy)-cyclopentyl]-N-thiazol-2-yl-propionamide (450 mg, 0.94 mmol) in ethanol (9.4 mL) was treated with pyridinium *p*-toluenesulfonate (24 mg, 0.094 mmol). The

resulting reaction mixture was heated at 60°C for 4 h. The reaction mixture was allowed to cool to 25°C and then concentrated *in vacuo*. The resulting yellow residue was diluted with ethyl acetate (100 mL) and then washed with a saturated aqueous sodium chloride solution. The organic layer was dried over magnesium sulfate, filtered, and concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 1/3 to 1/7 hexanes/ethyl acetate) afforded the 3-(3-hydroxy-cyclopentyl)-2-(4-methanesulfonyl-phenyl)-N-thiazol-2-yl-propionamide (318 mg, 86%) as a white foam: mp 69-72°C; FAB-HRMS m/e calcd for $C_{18}H_{22}N_2O_4S_2$ $(M+H)^+$ 395.1099, found 395.1091.

Example 38
2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-3-(3-hydroxy-cyclopentyl)-N-pyrazin-2-yl-propionamide



[0210] A solution of 1,1,1,3,3,3-hexamethyldisilazane (3.75 mL, 17.24 mmol) in freshly distilled tetrahydrofuran (50 mL) was treated slowly with a 2.3M solution of *n*-butyllithium in hexanes (7.0 mL, 16.1 mmol) at -45°C. The resulting reaction solution was stirred at -40°C for 45 min and then treated slowly with a solution of 2-(3-chloro-4-methylsulfanyl-phenyl)-N-[2(R)-hydroxy-1(R)-methyl-2(R)-phenyl-ethyl]-N-methyl-acetamide (prepared as in Example 30, 2.5 g, 6.87 mmol) in tetrahydrofuran (10 mL) via a cannula. A yellow solution was obtained, and the reaction was allowed to warm to 0°C, where it was stirred for 30 min. This reaction solution was cooled to -50°C and treated with 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (5 mL) followed by the dropwise addition of a solution of 2-(3-iodomethyl-cyclopentyloxy)-tetrahydropyran (prepared as in Example 35, 3.2 g, 10.3 mmol) in tetrahydrofuran (10 mL). After the addition, the reaction solution was warmed to 0°C, where it was stirred for 2 h, and then warmed to 25°C, where it was stirred for an additional 2 h. The reaction solution

was diluted with methylene chloride (100 mL) and washed with a saturated aqueous sodium chloride solution (100 mL). The organic layer was separated, dried over magnesium sulfate, filtered, and concentrated in *vacuo*. Flash chromatography (Merck Silica gel 60, 70-230 mesh, 40-60% ethyl acetate/hexanes) afforded 2(R)-(3-chloro-4-methylsulfanyl-phenyl)-N-(2(R)-hydroxy-1(R)-methyl-2(R)-phenyl-ethyl)-N-methyl-3-[3-(tetrahydro-pyran-2-yloxy)-cyclopentyl]-propionamide (3.4 g, 90.7%) as a white solid.

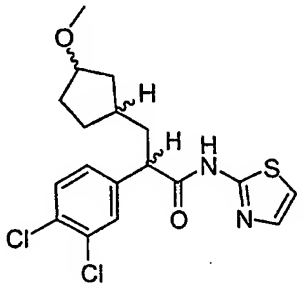
[0211] A solution of 2(R)-(3-chloro-4-methylsulfanyl-phenyl)-N-(2(R)-hydroxy-1(R)-methyl-2(R)-phenyl-ethyl)-N-methyl-3-[3-(tetrahydro-pyran-2-yloxy)-cyclopentyl]-propionamide (0.96 g, 1.758 mmol) in dioxane (20 mL) was treated with a 9N aqueous sulfuric acid solution (1.5 mL). The resulting reaction solution was heated under reflux for 15 h. In another flask, a solution of 2(R)-(3-chloro-4-methylsulfanyl-phenyl)-N-(2(R)-hydroxy-1(R)-methyl-2(R)-phenyl-ethyl)-N-methyl-3-[3-(tetrahydro-pyran-2-yloxy)-cyclopentyl]-propionamide (1g, 1.83 mmol) in dioxane (20 mL) and a 9N aqueous sulfuric acid solution (1.5 mL) was heated under reflux for 7 h. The two reactions were combined, diluted with methylene chloride (100 mL), and washed with a saturated aqueous sodium chloride solution (100 mL). The organic layer was separated, dried over magnesium sulfate, filtered, and concentrated in *vacuo*. Flash chromatography (Merck Silica gel 60, 70-230 mesh, 8-10% methanol/methylene chloride) afforded 2(R)-(3-chloro-4-methylsulfanyl-phenyl)-3-(3-hydroxy-cyclopentyl)-propionic acid (496 mg 43.9%) as an off-white foam.

[0212] A solution of 2(R)-(3-chloro-4-methylsulfanyl-phenyl)-3-(3-hydroxy-cyclopentyl)-propionic acid (350 mg, 1.11 mmol) in formic acid (35 mL) was treated with a 30% aqueous hydrogen peroxide solution (1 mL, 7.89 mmol) at 25°C. The mixture was then allowed to stir at 25°C overnight. The solvent was evaporated in *vacuo*. The resulting residue was azeotroped with toluene to remove water and then co-evaporated with *N,N*-dimethylformamide to remove formic acid to afford crude 2(R)-(3-chloro-4-methanesulfonyl-phenyl)-3-(3-formyloxy-cyclopentyl)-propionic acid (430 mg, 103%) as an off-white solid.

[0213] A solution of 2(R)-(3-chloro-4-methanesulfonyl-phenyl)-3-(3-formyloxy-cyclopentyl)-propionic acid (380 mg, 1.01 mmol) in toluene (10 mL) at 0°C was treated with *N,N*-dimethylformamide (0.008 mL) followed by a 2.0M solution of oxalyl chloride in methylene chloride (0.75 mL). The reaction mixture was allowed to stir at 0°C for 30 min and at 25°C for 1.5 h. It appeared that a thick oil in the bottom of the reaction flask never solubilized. Additional methylene chloride (10 mL) was added at 25°C followed by *N,N*-dimethylformamide (0.002 mL) and oxalyl chloride (0.3 mL). The reaction mixture was stirred at 25°C for an additional 45 min and then concentrated in *vacuo*. The residue was dissolved in dry tetrahydrofuran (5 mL) and cooled to 0°C. This cold solution was then treated with a solution of 2-aminopyrazine (142 mg, 1.5 mmol) and pyridine (0.121 mL, 1.5 mmol) in dry tetrahydrofuran (5 mL) via a cannula. The resulting reaction mixture was stirred at 0°C for 1 h and then diluted with methylene chloride (100 mL). The organic layer was successively washed with a 1M aqueous citric acid solution (2 x 100 mL), a saturated sodium bicarbonate solution (1 x 100 mL), and a saturated sodium chloride solution (1 x 100 mL). The organics were dried over magnesium sulfate, filtered, and concentrated in *vacuo* to afford formic acid 3-[2(R)-(3-chloro-4-methanesulfonyl-phenyl)-2-(pyrazin-2-ylcarbamoyl)-ethyl]-cyclopentyl ester (375 mg, 82%) as an off-white solid.

[0214] To a solution of formic acid 3-[2(R)-(3-chloro-4-methanesulfonyl-phenyl)-2-(pyrazin-2-ylcarbamoyl)-ethyl]-cyclopentyl ester (375 mg, 0.83 mmol) in methanol (50 mL) at 0°C was bubbled ammonia gas for 15 min. The resulting reaction solution was stirred at 0°C for 15 min. The solvent was removed in *vacuo*. Flash chromatography (Merck Silica gel 60, 70-230 mesh, 1-5% methanol/methylene chloride) afforded 2(R)-(3-chloro-4-methanesulfonyl-phenyl)-3-(3-hydroxy-cyclopentyl)-N-pyrazin-2-yl-propionamide (260 mg, 62.5%) as an off-white solid.

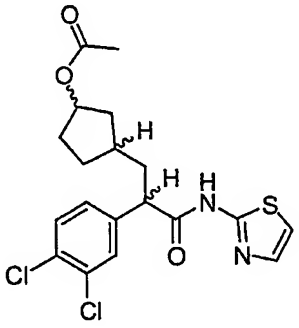
Example 39
2-(3,4-Dichloro-phenyl)-3-(3-methoxy-cyclopentyl)-N-thiazol-2-yl-propionamide



[0215] A solution of 2-(3,4-dichloro-phenyl)-3-(3-hydroxy-cyclopentyl)-propionic acid methyl ester (prepared as in Example 36, 194.1 mg, 0.61 mmol), silver carbonate (320.6 mg, 1.16 mmol), silver tetrafluoroborate (131.0 mg, 0.67 mmol), and iodomethane (72 μL, 1.16 mmol) in acetonitrile (6.1 mL) was stirred at 25°C for 48 h. The resulting reaction mixture was then heated under reflux for 24 h. The reaction mixture was then filtered through a pad of celite, and the celite pad was washed well with ethyl acetate. The filtrate was concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 10% ethyl acetate/hexanes) afforded 2-(3,4-dichloro-phenyl)-3-(3-methoxy-cyclopentyl)-propionic acid methyl ester (68.8 mg, 34%) as a yellow oil which was used without further purification.

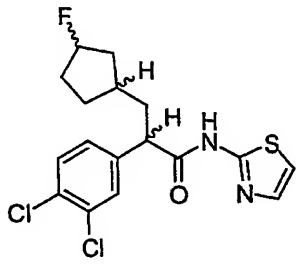
[0216] 2-(3,4-Dichloro-phenyl)-3-(3-methoxy-cyclopentyl)-propionic acid methyl ester (118.3 mg, 0.36 mmol) and 2-aminothiazole (35.8 mg, 0.36 mmol) were treated with a solution of magnesium methoxide in methanol (7.4 wt.%, 2.6 mL, 1.79 mmol). The resulting reaction mixture was then heated under reflux for 30 h. The reaction mixture was allowed to cool to 25°C and then filtered through celite. The celite was thoroughly washed with ethyl acetate, and the filtrate was concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 40% ethyl acetate/hexanes) afforded the 2-(3,4-dichloro-phenyl)-3-(3-methoxy-cyclopentyl)-N-thiazol-2-yl-propionamide (9.6 mg, 7%) as a white glass: $(ES)^+$-HRMS m/e calcd for $C_{18}H_{20}Cl_2N_2O_2S$ $(M+H)^+$ 399.0696, found 399.0700.

Example 40
Acetic acid 3-[2-(3,4-dichloro-phenyl)-2-(thiazol-2-ylcarbamoyl)-ethyl]-cyclopentyl ester



[0217] A solution of 2-(3,4-dichloro-phenyl)-3-(3-hydroxy-cyclopentyl)-N-thiazol-2-yl-propionamide (prepared as in Example 36, 147.8 mg, 0.38 mmol) and acetic anhydride (72 μL, 0.77 mmol) in pyridine (2 mL) and methylene chloride (1 mL) was stirred at 25°C for 20 h. The resulting reaction mixture was concentrated *in vacuo*. Since the reaction did not go to completion, the residue was re-dissolved in pyridine (1.8 mL) and acetic anhydride (1.4 mL, 14.84 mmol) and then stirred at 25°C for an additional 7 h. The reaction mixture was concentrated *in vacuo* and then diluted with ethyl acetate (50 mL). The organic layer was washed with a 1N aqueous hydrochloric acid solution (3 x 50 mL), water (50 mL), and a saturated aqueous sodium chloride solution (50 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 35% ethyl acetate/hexanes) afforded acetic acid 3-[2-(3,4-dichloro-phenyl)-2-(thiazol-2-ylcarbamoyl)-ethyl]-cyclopentyl ester (80.8 mg, 49%) as a white solid: mp 49-52°C; $(ES)^+$-HRMS m/e calcd for $C_{19}H_{20}Cl_2N_2O_3S$ $(M+H)^+$ 427.0645 found 427.0648.

Example 41
2-(3,4-Dichloro-phenyl)-3-(3-fluoro-cyclopentyl)-N-thiazol-2-yl-propionamide

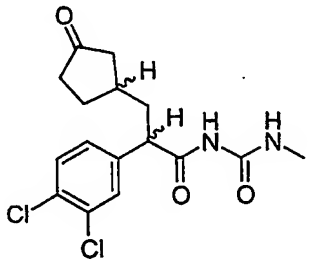


[0218] A solution of 2-(3,4-dichloro-phenyl)-3-(3-hydroxy-cyclopentyl)-propionic acid methyl ester (prepared as in Example 36, 180 mg, 0.56 mmol) in methylene chloride (0.28 mL) was treated with diethylaminosulfur trifluoride (0.11 mL, 0.85 mmol). The resulting mixture was stirred at 25°C for 18 h. At this time, the reaction was diluted with water (50 mL). This solution was extracted with methylene chloride (3 x 30 mL). The organics were dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 50/50 hexanes/ethyl acetate) afforded 2-(3,4-dichloro-phenyl)-3-(3-fluoro-cyclopentyl)-propionic acid methyl ester (71.2 mg, 39.3%) as a light yellow oil.

[0219] A mixture of 2-(3,4-dichloro-phenyl)-3-(3-fluoro-cyclopentyl)-propionic acid methyl ester (70 mg, 0.24 mmol) and 2-aminothiazole (26.3 mg, 0.26 mmol) in a solution of magnesium methoxide in methanol (7.4 wt.%, 0.63 mL, 0.43 mmol) was heated to 110°C for 18 h. At this time, the reaction mixture was cooled to 25°C and then concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 70/30 hexanes/ethyl acetate) afforded 2-(3,4-dichloro-phenyl)-3-(3-fluoro-cyclopentyl)-N-thiazol-2-yl-propionamide (2.3 mg, 2.7%) as a light yellow oil: EI-HRMS m/e calcd for $C_{17}H_{17}Cl_2N_2OS$ $(M+H)^+$ 387.0496, found 387.0499.

Example 42

1-[2-(3,4-Dichloro-phenyl)-3-(3-oxo-cyclopentyl)-propionyl]-3-methyl-urea

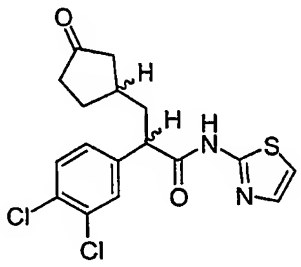


[0220] A solution of 1-[2-(3,4-dichloro-phenyl)-3-(3-hydroxy-cyclopentyl)-propionyl]-3-methyl-urea (prepared as in Example 35, 60.5 mg, 0.17 mmol) in methylene chloride (1.68 mL) was treated with pyridinium chlorochromate (20

wt.% on basic alumina, 218 mg, 0.20 mmol). The reaction mixture was stirred at 25°C for 4 h. At this time, the reaction was filtered through a plug of celite (ethyl acetate as eluent). The filtrate was concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 90/10 methylene chloride/methanol) afforded 1-[2-(3,4-dichloro-phenyl)-3-(3-oxo-cyclopentyl)-propionyl]-3-methyl-urea (45.8 mg, 76.1%) as a white solid: mp 70-74°C; FAB-HRMS m/e calcd for $C_{16}H_{18}Cl_2N_2O_3$ $(M+H)^+$ 357.0773, found 357.0768.

Example 43

2-(3,4-Dichloro-phenyl)-3-(3-oxo-cyclopentyl)-N-thiazol-2-yl-propionamide



[0221] A solution of 3-iodomethyl-cyclopentanone (prepared as in Example 35, 23.47 g, 0.10 mol), 1,3-propanediol (39.86 g, 0.52 mol), trimethyl orthoformate (13.61 g, 0.1257 mol), and *p*-toluenesulfonic acid monohydrate in benzene (524 mL) was heated under reflux for 6 h. The reaction was cooled to 25°C, diluted with water (1 L), and extracted with diethyl ether (2 x 400 mL). The organic layers were dried over magnesium sulfate, filtered, and concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 70-230 mesh, 15% diethyl ether/petroleum ether) afforded 2-iodomethyl-6,10-dioxa-spiro[4.5]decane (18.77 g, 63%) as a yellow oil.

[0222] A solution of diisopropylamine (2.5 mL, 17.80 mmol) in dry tetrahydrofuran (26 mL) and 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (8 mL) was cooled to -78°C under nitrogen and then treated with a 2.5M solution of *n*-butyllithium in hexanes (7.1 mL, 17.80 mmol). The reaction mixture was stirred at -78°C for 45 min and then treated dropwise with a solution of (3,4-dichloro-phenyl)-acetic acid methyl ester (prepared as in Example 1, 3.00 g, 13.69 mmol) in dry tetrahydrofuran (26 mL) and 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-

pyrimidinone (8 mL). The reaction mixture turned dark yellow in color and was allowed to stir at -78°C for 10 min and then at 0°C for 30 min. The reaction mixture was then cooled to -78°C, at which time, a solution of 2-iodomethyl-6,10-dioxa-spiro[4.5]decane (5.79 g, 20.54 mmol) in dry tetrahydrofuran (13 mL) and 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (8 mL) was added dropwise. The reaction mixture stirred at -78°C for 15 min, and was then allowed to warm to 25°C, where it was stirred for 20 h. The reaction mixture was quenched with a saturated aqueous ammonium chloride solution (100 mL) and then concentrated *in vacuo* to remove tetrahydrofuran. The aqueous residue was extracted with ethyl acetate (2 x 150 mL). The combined organic extracts were washed with a saturated aqueous lithium chloride solution (2 x 200 mL), water (200 mL) and a saturated aqueous sodium chloride solution (200 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 15% ethyl acetate/hexanes) afforded 2-(3,4-dichloro-phenyl)-3-(6,10-dioxa-spiro[4.5]dec-2-yl)-propionic acid methyl ester (3.92 g, 77%) as a yellow oil.

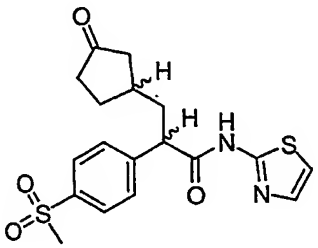
[0223] 2-(3,4-Dichloro-phenyl)-3-(6,10-dioxa-spiro[4.5]dec-2-yl)-propionic acid methyl ester (1.00 g, 2.68 mmol) was treated with a solution of magnesium methoxide in methanol (7.4 wt.%, 19 mL, 13.39 mmol). The reaction mixture was then treated with 2-aminothiazole (348.7 mg, 3.48 mmol). The resulting reaction mixture was then heated under reflux for 28 h. The reaction mixture was allowed to cool to 25°C and then filtered through celite. The celite was thoroughly washed with ethyl acetate, and the filtrate was concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 25-50% ethyl acetate/hexanes) afforded crude 2-(3,4-dichloro-phenyl)-3-(6,10-dioxa-spiro[4.5]dec-2-yl)-N-thiazol-2-yl-propionamide (768.5 mg) as a yellow oil which was used without further purification.

[0224] A solution of crude 2-(3,4-dichloro-phenyl)-3-(6,10-dioxa-spiro[4.5]dec-2-yl)-N-thiazol-2-yl-propionamide (768.5 mg, 1.74 mmol) in tetrahydrofuran (8.7 mL) and a 5% aqueous hydrochloric acid solution (3.9 mL) was stirred at 25°C for 64 h. The resulting reaction solution was concentrated *in vacuo* to remove

tetrahydrofuran and then diluted with ethyl acetate (100 mL). The organic layer was washed with a 1% aqueous hydrochloric acid solution (50 mL), water (50 mL) and a saturated aqueous sodium chloride solution (50 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 50% ethyl acetate/hexanes) afforded 2-(3,4-dichloro-phenyl)-3-(3-oxo-cyclopentyl)-N-thiazol-2-yl-propionamide (481.9 mg, 47% over 2 steps) as a white solid: mp 146-148°C; $(ES)^+$-HRMS m/e calcd for $C_{17}H_{16}Cl_2N_2O_2S$ $(M+H)^+$ 383.0383 found 383.0385.

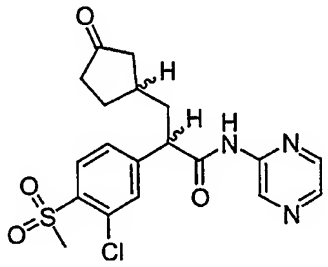
Example 44

2-(4-Methanesulfonyl-phenyl)-3-(3-oxo-cyclopentyl)-N-thiazol-2-yl-propionamide



[0225] A solution of 3-(3-hydroxy-cyclopentyl)-2-(4-methanesulfonyl-phenyl)-N-thiazol-2-yl-propionamide (prepared as in Example 37, 155 mg, 0.393 mmol) in methylene chloride (3 mL) was treated with pyridinium chlorochromate (20 wt.% on basic alumina, 508 mg, 0.471 mmol). The resulting reaction mixture was stirred at 25°C for 3 h, at which time, thin layer chromatography indicated a small amount of the 3-(3-hydroxy-cyclopentyl)-2-(4-methanesulfonyl-phenyl)-N-thiazol-2-yl-propionamide. The reaction mixture was then treated with an additional amount of pyridinium chlorochromate (20 wt.% on basic alumina, 127 mg, 0.118 mmol). The reaction mixture was allowed to stir at 25°C for 1 h and then filtered through a pad of celite. The pad of celite was washed well with ethyl acetate until the washings indicated the absence of product by thin layer chromatography. The filtrate was then concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 1/3 hexanes/ethyl acetate to 100% ethyl acetate) afforded 2-(4-methanesulfonyl-phenyl)-3-(3-oxo-cyclopentyl)-N-thiazol-2-yl-propionamide (46 mg, 30%) as a white foam: mp 94-97°C; FAB-HRMS m/e calcd for $C_{18}H_{20}N_2O_4S_2$ $(M+H)^+$ 393.0943, found 393.0948.

Example 45
2-(3-Chloro-4-methanesulfonyl-phenyl)-3-(3-oxo-cyclopentyl)-N-pyrazin-2-yl-propionamide



[0226] A solution of diisopropylamine (1.9 mL, 13.64 mmol) in dry tetrahydrofuran (19.5 mL) and 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (6.5 mL) cooled to -78°C under nitrogen was treated with a 2.5M solution of *n*-butyllithium in hexanes (5.5 mL, 13.64 mmol). The reaction mixture was stirred at -78°C for 45 min and then treated dropwise with a solution of (3-chloro-4-methylsulfanyl-phenyl)-acetic acid methyl ester (prepared as in Example 4, 2.42 g, 10.49 mmol) in dry tetrahydrofuran (19.5 mL) and 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (6.5 mL). The reaction mixture turned bright yellow. The reaction mixture was allowed to stir at -78°C for 15 min, then at 0°C for an additional 30 min, and then re-cooled to -78°C. At this point, a solution of 2-iodomethyl-6,10-dioxa-spiro[4.5]decane (prepared as in Example 43, 3.85 g, 13.64 mmol) in dry tetrahydrofuran (10 mL) and 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (3 mL) was added dropwise. The reaction mixture stirred at -78°C for 30 min and was then allowed to warm to 25°C, where it was stirred for 24 h. The reaction mixture was concentrated *in vacuo* to remove tetrahydrofuran. The aqueous residue was diluted with ethyl acetate (200 mL), and the organic layer was washed with a saturated aqueous lithium chloride solution (2 x 200 mL), water (200 mL), and a saturated aqueous sodium chloride solution. The organic layer was dried over magnesium sulfate, filtered, and concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 25% ethyl acetate/hexanes) afforded the 2-(3-chloro-4-methylsulfanyl-phenyl)-3-(6,10-dioxa-spiro[4.5]dec-2-yl)-propionic acid methyl ester (2.27 g, 56%) as a yellow oil: EI-HRMS m/e calcd for $C_{19}H_{25}ClO_4S$ $(M)^+$ 384.1162 found 384.1181.

[0227] A solution of 2-(3-chloro-4-methylsulfanyl-phenyl)-3-(6,10-dioxa-spiro[4.5]dec-2-yl)-propionic acid methyl ester (1.18 g, 3.07 mmol) in methylene chloride (8 mL) cooled to 0°C was slowly treated with a mixture of 3-chloroperoxybenzoic acid (~70%, 1.51 g based on 70%, 6.13 mmol) and sodium bicarbonate (1.03 g, 12.26 mmol) in methylene chloride (8 mL). The thick reaction mixture was diluted with methylene chloride (8 mL) and was then allowed to warm to 25°C, where it was stirred for 4 h. The reaction mixture was diluted with methylene chloride (200 mL) and water (200 mL). The organic phase was cooled down to 0°C and then slowly treated with a saturated aqueous sodium bisulfite solution (200 mL). The organic layer was washed with a saturated aqueous sodium bicarbonate solution (100 mL), water (100 mL), and a saturated aqueous sodium chloride solution (100 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 70-230 mesh, 50% ethyl acetate/hexanes) afforded 2-(3-chloro-4-methanesulfonyl-phenyl)-3-(6,10-dioxa-spiro[4.5]dec-2-yl)-propionic acid methyl ester (1.22 g, 95%) as a colorless oil: EI-HRMS m/e calcd for $C_{19}H_{25}ClO_6S$ $(M)^+$ 416.1060 found 416.1054.

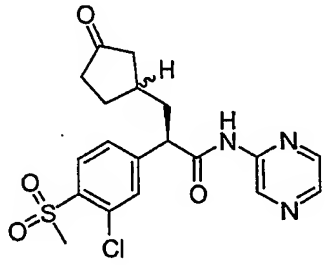
[0228] A solution of 2-(3-chloro-4-methanesulfonyl-phenyl)-3-(6,10-dioxa-spiro[4.5]dec-2-yl)-propionic acid methyl ester (1.21 g, 2.90 mmol) in tetrahydrofuran (14.5 mL) was treated with a 5% aqueous hydrochloric acid solution (6.4 mL) and stirred at 25°C for 29 h. The reaction was then concentrated *in vacuo* to remove tetrahydrofuran. The resulting aqueous residue was diluted with water (200 mL) and extracted with ethyl acetate (2 x 100 mL). The combined organic layers were dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 70-230 mesh, 50% ethyl acetate/hexanes) afforded 2-(3-chloro-4-methanesulfonyl-phenyl)-3-(3-oxo-cyclopentyl)-propionic acid methyl ester (1.03 g, 99%) as a colorless oil: EI-HRMS m/e calcd for $C_{16}H_{19}ClO_5S$ $(M)^+$ 358.0642 found 358.0630.

[0229] A solution of 2-(3-chloro-4-methanesulfonyl-phenyl)-3-(3-oxo-cyclopentyl)-propionic acid methyl ester (1.02 g, 2.84 mmol) in methanol (7.1 mL) was treated

with a 1N aqueous sodium hydroxide solution (6.0 mL, 6.0 mmol). The reaction mixture was stirred at 25°C for 16 h. The reaction mixture was then concentrated *in vacuo* to remove methanol. The resulting aqueous residue was diluted with water (100 mL) and acidified to pH=3 with a 1N aqueous hydrochloric acid solution then extracted with ethyl acetate (2 x 150 mL). The combined organic layers were washed with a saturated aqueous sodium chloride solution (100 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo*. The resulting yellow oily solid was triturated with ethyl acetate/petroleum ether to afford 2-(3-chloro-4-methanesulfonyl-phenyl)-3-(3-oxo-cyclopentyl)-propionic acid (800.0 mg, 82%) as a white solid: mp 164-167°C; $(ES)^+$-HRMS m/e calcd for $C_{15}H_{17}ClO_5S$ $(M+H)^+$ 345.0558 found 345.0561.

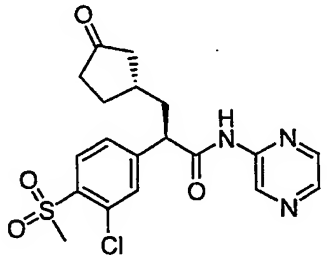
[0230] A solution of 2-(3-chloro-4-methanesulfonyl-phenyl)-3-(3-oxo-cyclopentyl)-propionic acid (594.8 mg, 1.725 mmol) in methylene chloride (8.6 mL) and *N,N*-dimethylformamide (2 drops) cooled to 0°C was treated with oxalyl chloride (226 μL, 2.587 mmol). The reaction mixture was stirred at 0°C for 15 min and then stirred at 25°C for 2 h. The reaction mixture was concentrated *in vacuo* to afford a red oil. This red oil was dissolved in tetrahydrofuran (4.03 mL), cooled to 0°C, and then slowly treated with a solution of 2-aminopyrazine (246.1 mg, 2.587 mmol) and pyridine (209 μL, 2.587 mmol) in tetrahydrofuran (4.3 mL). The resulting reaction mixture was stirred at 0°C for 15 min and then stirred at 25°C for 2 h. The reaction mixture was quenched with a 1N aqueous citric acid solution (20 mL) and then extracted with ethyl acetate (2 x 100 mL). The combined organic layers were washed with a saturated aqueous sodium bicarbonate solution (2 x 100 mL) and a saturated aqueous sodium chloride solution (100 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 80% ethyl acetate/hexanes) afforded 2-(3-chloro-4-methanesulfonyl-phenyl)-3-(3-oxo-cyclopentyl)-N-pyrazin-2-yl-propionamide (207.3 mg, 28%) as a pale yellow foam: mp 93-96°C (foam to gel); $(ES)^+$-HRMS m/e calcd for $C_{19}H_{20}ClN_3O_4S$ $(M+H)^+$ 422.0936 found 422.0938.

Example 46
2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-3-(3-oxo-cyclopentyl)-N-pyrazin-2-yl-propionamide



[0231] A solution of 2(R)-(3-chloro-4-methanesulfonyl-phenyl)-3-(3-hydroxy-cyclopentyl)-N-pyrazin-2-yl-propionamide (prepared as in Example 38, 60 mg, 0.142 mmol) in methylene chloride (10 mL) was treated with Dess-Martin periodinane (132.5 mg, 0.312 mmol). The reaction mixture was stirred at 25°C for 2 h. The reaction mixture was then diluted with methylene chloride (10 mL) and washed with a 1M aqueous citric acid solution (10 mL). The pH of the aqueous layer was adjusted to 5. The aqueous layer was then extracted with methylene chloride (20 mL). The combined organic layers were dried over magnesium sulfate, filtered, and concentrated in *vacuo*. Flash chromatography (Merck Silica gel 60, 70-230 mesh, 50-100% ethyl acetate/hexanes) afforded 2(R)-(3-chloro-4-methanesulfonyl-phenyl)-3-(3-oxo-cyclopentyl)-N-pyrazin-2-yl-propionamide (45 mg, 75%) as an off-white solid.

Example 47
2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-3-((S)-3-oxo-cyclopentyl)-N-pyrazin-2-yl-propionamide



Step 1

[0232] A solution of (S,S)-hydrobenzoin (10.0 g, 46.7 mmol), pyridinium *p*-toluene sulfonate (1.4 g, 5.6 mmol), 2-cyclopentene-1-one (20 mL, 238.7 mmol) and cyclohexane (200 mL) was heated under reflux for 4 h with a Dean-Stark trap to remove water formed during the reaction. The mixture was then cooled in an ice bath for 20 min, and the insolubles were filtered off through filter aid. The resulting solution was washed with a 10% aqueous potassium bicarbonate solution (2 x 25 mL) and water (2 x 25 mL), and then dried over anhydrous sodium sulfate. The dried solution was passed through a pad of silica gel 60 (26 g of 230-400 mesh). The pad was further eluted with additional hexanes (475 mL). The resulting solution was concentrated *in vacuo* to afford 2(S),3(S)-diphenyl-1,4-dioxa-spiro[4.4]non-6-ene (10.55 g, 81%) as a white solid.

Step 2

[0233] A solution of 1,2-dichloroethane (85 mL) and diethyl zinc (6.5 mL, 63.4 mmol) cooled to -15°C under a nitrogen atmosphere was treated with iodochloromethane (8.9 mL, 122 mmol), via syringe, at such a rate as to allow the temperature to rise to -7°C (over about 5 min). The mixture was stirred with cooling for an additional 15 min at -7°C to -13°C until there was no further exotherm. The reaction was then stirred at ~0°C for 10 min, then re-cooled to -35°C. At this time, a solution of 2(S),3(S)-diphenyl-1,4-dioxa-spiro[4.4]non-6-ene (8.5 g, 30.5 mmol) in 1,2-dichloroethane (51 mL) was added via syringe over 10 min. The reaction temperature increased to -26°C during the addition. The reaction was then stirred at -26°C for 30 min. At this time, the reaction was re-cooled to -35°C and a 10% aqueous potassium bicarbonate solution (17 mL) was added dropwise while maintaining the temperature below -12°C. The cooling bath was then removed, and the reaction was allowed to warm to 25°C, where it was stirred for 1.5 h. The liquid was decanted, and the white pasty residue was slurried with *tert*-butyl methyl ether (2 x 100 ml). The supernatants were decanted, and the combined organic solution was washed with a 10% aqueous potassium bicarbonate solution (2 x 100 mL) and a 20% aqueous sodium chloride solution (1 x 100 mL), and then dried over anhydrous sodium sulfate. The solvent was removed *in vacuo* to give 1(R),5(S)-bicyclo[3.1.0]hexan-2-spiro-2'-(4(S),5(S)-

diphenyl dioxolane) (8.91 g, 99%) as a white solid. HPLC (Chiralpak AD-RH, 150x5 mm, 220 nm, 0.5 cc/min, 80 min, mobile phase 5 ethanol/5 methanol/4 water, R_t 1(R),5(S) = 20.24 min, 1(S), 5(R) = 27.83 min) indicated 87 area% purity and 91% de.

Step 3

[0234] A mixture of 1(R),5(S)-bicyclo[3.1.0]hexan-2-spiro-2'-(4(S),5(S)-diphenyl dioxolane (2.51 g, 8.58 mmol) and anhydrous potassium carbonate (2.99 g, 21.6 mmol) in methylene chloride (25 mL) was cooled to -8°C under nitrogen and then was treated with trimethyl silyl iodide (1.52 mL, 10.7 mmol) via syringe over 7 min. The reaction was stirred for 1 h at -6°C to -8°C. At this time, a 30% aqueous sodium thiosulfate solution (25 mL, 30 mmol) was added to the cold reaction over 5 min. When the exotherm subsided, the bath was removed, and the reaction was stirred for 1.5 h at 25°C. The layers were separated, and the aqueous phase was extracted with methylene chloride (25 mL). The combined extracts were washed with water (2 x 25 mL), dried over anhydrous sodium sulfate, and concentrated *in vacuo* to give a crude product (3.34 g, 92.7%) as a white solid. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 2% ethyl ether/petroleum ether) afforded 7(R)-iodomethyl-2(S),3(S)-diphenyl-1,4-dioxa-spiro[4.4]nonane (2.445 g, 67.7%) as a white solid.

Step 4

[0235] A solution of 1,1,1,3,3,3-hexamethyldisilazane (3.18 mL, 15.05 mmol) in tetrahydrofuran (20 mL) cooled to -20°C was treated with a 2.5M solution of *n*-butyllithium in hexanes (5.8 mL, 14.5 meq) over 10 min at such a rate as to keep the temperature below -15°C. The mixture was stirred for an additional 5 min. The cold reaction mixture was then treated with a solution of 2-(3-chloro-4-methylsulfanyl-phenyl)-N-(2(R)-hydroxy-1(R)-methyl-2-phenyl-ethyl)-N-methylacetamide (prepared as in Example 30, 2.77 g, 7.61 mmol) in tetrahydrofuran (22 mL) via syringe over 8 min, at such a rate as to keep the temperature below -15°C. The reaction was then slowly allowed to warm to -7°C over 20 min and then a solution of 7(R)-iodomethyl-2(S),3(S)-diphenyl-1,4-

dioxa-spiro[4.4]nonane (2.42 g, 5.76 mmol) and 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (1.7 mL, 14.1 mmol) in tetrahydrofuran (10 mL) was added over 6 min. An exotherm increased the temperature to -4°C during the addition. The reaction was allowed to warm to 0°C, where it was stirred for an additional 3 h. At this time, the reaction was poured into a mixture of toluene (70 mL) and a 20% aqueous ammonium chloride solution (50 mL, 187 mmol) and was then stirred vigorously. The organic layer was separated and re-washed with a 20% aqueous ammonium chloride solution (50 mL, 187 mmol). The organic layer was dried over anhydrous sodium sulfate, and concentrated *in vacuo* to give 2(R)-(3-chloro-4-methylsulfanyl-phenyl)-3-(2(S),3(S)-diphenyl-1,4-dioxa-spiro[4.4]non-7(S)-yl)-N-(2(R)-hydroxy-1(R)-methyl-2-phenyl-ethyl)-N-methyl-propionamide (3.86 g) as an overweight brown semi-solid. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 1/1 hexanes/ethyl acetate) afforded 2(R)-(3-chloro-4-methylsulfanyl-phenyl)-3-(2(S),3(S)-diphenyl-1,4-dioxa-spiro[4.4]non-7(S)-yl)-N-(2(R)-hydroxy-1(R)-methyl-2-phenyl-ethyl)-N-methylpropionamide as a yellow foam (2.28 g, 60.4%).

Step 5

[0236] A mixture of 2(R)-(3-chloro-4-methylsulfanyl-phenyl)-3-(2(S),3(S)-diphenyl-1,4-dioxa-spiro[4.4]non-7(S)-yl)-N-(2(R)-hydroxy-1(R)-methyl-2-phenyl-ethyl)-N-methyl-propionamide (2.28 g, 3.47 mmol), 1,4-dioxane (4.56 mL), and a 9N aqueous sulfuric acid solution (4.5 mL) was heated under reflux for 18 h. At this time, the reaction was cooled to 5°C and diluted with water (12 mL). The aqueous portion was decanted. The resulting viscous oil was dissolved in *tert*-butyl methyl ether (30 mL), washed with water (3 x 10 mL), and dried over anhydrous sodium sulfate. The combined aqueous phases were back-extracted with ethyl acetate (20 mL). The combined organics were washed with water (3 x 10 mL), dried over anhydrous sodium sulfate, and concentrated *in vacuo* to afford the crude product (1.76 g) as an overweight, brown semi-solid. This material was recrystallized in 1:1 ethyl acetate:hexanes to afford 2(R)-(3-chloro-4-methylsulfanyl-phenyl)-3-((S)-3-oxo-cyclopenyl)-propionic acid (422.1 mg, 38.8%) as an off-white solid. HPLC (Zorbax XDB-C8 150x5mm, 20-100% acetonitrile/water over 20 min, 20 min run, 220 nm, 1 cc/min R_t = 10.3 min) indicated 98.5 area% purity, and HPLC

(Chiralpak AD-RH, 150x5 mm, 70% ethanol/water, 30 min run, 230 nm, 0.5 cc/min, R_t of 2(R), 3(S) = 15.2 min; 2(R), 3(R) = 22.1 min) showed 90% de.

Step 6

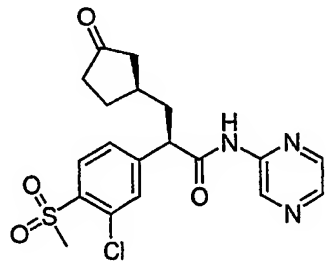
[0237] A solution of 2(R)-(3-chloro-4-methylsulfanyl-phenyl)-3-((S)-3-oxo-cyclopenyl)-propionic acid (189 mg, 0.598 mmol) and acetone (5 mL) stirred at 25°C was treated with a 0.05M solution of dimethyl dioxirane in acetone (26 mL, 1.3 mmol. After 1 h, the volatiles were removed *in vacuo* to afford 2(R)-(3-chloro-4-methanesulfonyl-phenyl)-3-((S)-3-oxo-cyclopentyl)-propionic acid (172.4 mg, 83%) as a white solid.

Step 7

[0238] A mixture of 2(R)-(3-chloro-4-methanesulfonyl-phenyl)-3-((S)-3-oxo-cyclopentyl)-propionic acid (544 mg, 1.58 mmol), methylene chloride (7 mL), toluene (5 mL), and a catalytic amount of *N,N*-dimethylformamide (10 µL, 0.13 mmol) under nitrogen was treated with oxalyl chloride (1.4 mL, 16.3 mmol). The reaction was stirred for 1 h (gas evolution). At this time, the reaction was then partially evaporated *in vacuo* at 25°C to remove excess oxalyl chloride. The residue was co-evaporated with toluene (2 x 4 mL) and concentrated *in vacuo* to about 4 mL. The resulting brown slurry was added via a cannula over 15 min to a mixture of 2-aminopyrazine (189 mg, 1.99 mmol), methylene chloride (4 mL), and pyridine (170 µL, 2.1 mmol) cooled to -10°C. The reaction was allowed to warm to 25°C, where it was stirred for 18 h. At this time, the reaction was quenched with water (700 µL) followed by a 1M aqueous citric acid solution (5 ml, 5 mmol). The reaction then was diluted with ethyl acetate (40 ml) and a 1M aqueous citric acid solution (20 mL), mixed well, and the phases were separated. The organic phase was washed with a 1M aqueous citric acid solution (3 x 5 mL). The combined aqueous phases were back-extracted with ethyl acetate (30 mL). The combined organic phases were washed with a 10% aqueous potassium bicarbonate solution (3 x 25 mL), dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo* to afford a crude product (506.6 mg, 76%) as a brown foam. Flash chromatography (Merck Silica gel 60, 230-400 mesh, ethyl acetate) afforded 2(R)-(3-chloro-4-methanesulfonyl-phenyl)-3-((S)-3-oxo-cyclopentyl)-N-

pyrazin-2-yl-propionamide (442 mg, 66 %) as a tan foam. HPLC (Zorbax XDB-C8 150x5mm, 20-100% acetonitrile/water over 20 min, 20 min run, 220 nm, 1 cc/min R_t = 7.7 min) indicated 95.3 area % purity, and HPLC (Chiralpak AD-RH, 150x5 mm, 5 ethanol/5 methanol/4 water, 60 min run, 220 nm, 0.5 cc/min, R_t of 2(R), 3(S) = 25.2 min; 2(R), 3(R) = 39.2 min) showed 90.5% de.

Example 48
2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-3-((R)-3-oxo-cyclopentyl)-N-pyrazin-2-yl-propionamide



[0239] 2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-3-((R)-3-oxo-cyclopentyl)-N-pyrazin-2-yl-propionamide was prepared in a similar manner as 2(R)-(3-chloro-4-methanesulfonyl-phenyl)-3-((S)-3-oxo-cyclopentyl)-N-pyrazin-2-yl-propionamide (Example 47) starting with (R,R)-hydrobenzoin (Wang, Z.-M.; Sharpless, K. B. *J. Org. Chem.* **1994**, *59*, 8302).

Step 1

[0240] 2(R),3(R)-diphenyl-1,4-dioxa-spiro[4.4]non-6-ene was similarly obtained from 2-cyclopentene-1-one and (R,R)-hydrobenzoin in toluene in 91.5% yield as a white solid.

Step 2

[0241] 1(S),5(R)-bicyclo[3.1.0]hexan-2-spiro-2'-(4(R),5(R)-diphenyl dioxolane) was similarly obtained from 2(R),3(R)-diphenyl-1,4-dioxa-spiro[4.4]non-6-ene in 68% yield after crystallization from pentane. HPLC (Zorbax XDB-C8 150x5mm, 5-100% acetonitrile/water + 0.1% trifluoroacetic acid, R_t 18.0 min) indicated 96.5 area% purity.

Step 3

[0242] 7(S)-iodomethyl-2(R),3(R)-diphenyl-1,4-dioxa-spiro[4.4]nonane was similarly obtained from 1(S),5(R)-bicyclo[3.1.0]hexan-2-spiro-2'-(4(R),5(R)-diphenyl dioxolane) at 25°C in 71.7% yield as a white solid.

Step 4

[0243] 2(R)-(3-chloro-4-methylsulfanyl-phenyl)-3-(2(R),3(R)-diphenyl-1,4-dioxa-spiro[4.4]non-7(R)-yl)-N-(2(R)-hydroxy-1(R)-methyl-2-phenyl-ethyl)-N-methylpropionamide was similarly obtained from 7(S)-iodomethyl-2(R),3(R)-diphenyl-1,4-dioxa-spiro[4.4]nonane in 65% yield.

Step 5

[0244] 2(R)-(3-chloro-4-methylsulfanyl-phenyl)-3-((R)-3-oxo-cyclopenyl)-propionic acid was similarly obtained from 2(R)-(3-chloro-4-methylsulfanyl-phenyl)-3-(2(R),3(R)-diphenyl-1,4-dioxa-spiro[4.4]non-7(R)-yl)-N-(2(R)-hydroxy-1(R)-methyl-2-phenyl-ethyl)-N-methylpropionamide in 72% yield as a yellow foam.

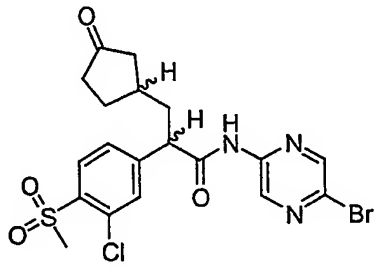
Step 6

[0245] 2(R)-(3-chloro-4-methanesulfonyl-phenyl)-3-((R)-3-oxo-cyclopentyl)-propionic acid was similarly obtained from 2(R)-(3-chloro-4-methylsulfanyl-phenyl)-3-((R)-3-oxo-cyclopenyl)-propionic acid in 90% yield as a white solid.

Step 7

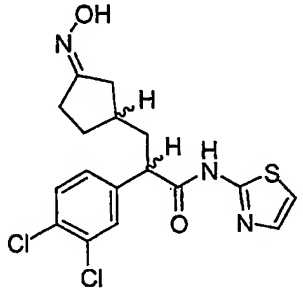
[0246] 2(R)-(3-chloro-4-methanesulfonyl-phenyl)-3-((R)-3-oxo-cyclopentyl)-N-pyrazin-2-yl-propionamide was similarly obtained from 2(R)-(3-chloro-4-methanesulfonyl-phenyl)-3-((R)-3-oxo-cyclopentyl)-propionic acid in 43% yield as a white foam. HPLC (Chiralpak AD-RH, 150x5 mm, 5 ethanol/5 methanol/4 water, 60 min run, 220 nm, 0.5 cc/min, R_t of 2(R), 3(S) = 25.4 min; 2(R), 3(R) = 38.9 min) indicated 95.9 area% purity and >99% de.

Example 49
N-(5-Bromo-pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(3-oxo-cyclopentyl)-propionamide



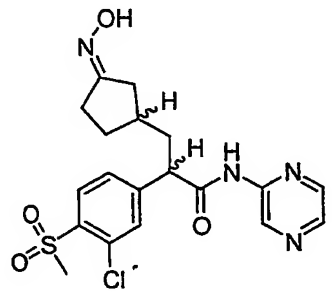
[0247] A solution of 2-(3-chloro-4-methanesulfonyl-phenyl)-3-(3-oxo-cyclopentyl)-propionic acid (prepared as in Example 45, 1.05 g, 3.04 mmol) and *N,N*-dimethylformamide (5 drops) in methylene chloride (10 mL) cooled to 0°C was treated with oxalyl chloride (0.39 mL, 4.57 mmol). The reaction mixture was stirred at 0°C for 1 h and then at 25°C for 1 h. The solution was then concentrated *in vacuo*, and the orange-brown gel was dissolved in methylene chloride (5 mL). The resulting solution was added dropwise via an addition funnel at 0°C to a solution of 2-amino-5-bromopyrazine (0.79 g, 4.57 mmol, prepared according to *Tetrahedron* **1988**, *44*, 2977-2983) in methylene chloride (5 mL) and pyridine (0.37 mL, 4.57 mmol). The reaction mixture was stirred at 0°C for 30 min and then at 25°C for 3 h. The reaction mixture was quenched with a 1N aqueous citric acid solution (10 mL) and stirred for 15 min. The reaction was then diluted with a 1N aqueous citric acid solution (50 mL) and ethyl acetate (75 mL). The organic layer was washed with a saturated aqueous sodium bicarbonate solution (50 mL), water (50 mL), and a saturated aqueous sodium chloride solution (50 mL), dried over magnesium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 40M, Silica, 50% ethyl acetate/hexanes) afforded N-(5-bromo-pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(3-oxo-cyclopentyl)-propionamide (0.622 g, 41%) as a yellow-orange foam: mp 97-103°C (foam to gel); $(ES)^+$-HRMS m/e calcd for $C_{19}H_{19}BrClN_3O_4S$ $(M+H)^+$ 500.0041 found 500.0048.

Example 50
2-(3,4-Dichloro-phenyl)-3-(3-hydroxyimino-cyclopentyl)-N-thiazol-2-yl-propionamide



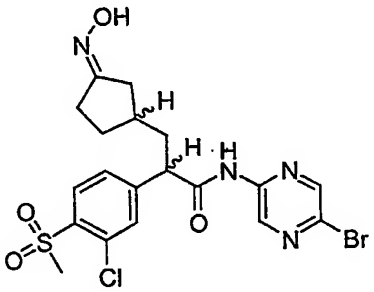
[0248] A solution of the 2-(3,4-dichloro-phenyl)-3-(3-oxo-cyclopentyl)-N-thiazol-2-yl-propionamide (prepared as in Example 43, 144.6 mg, 0.38 mmol) and hydroxylamine hydrochloride (39.7 mg, 0.57 mmol) in methanol (1.1 mL) and pyridine (1.1 mL) was heated under reflux for 3 h. The reaction mixture was allowed to cool to 25°C and then was concentrated *in vacuo*. The resulting residue was diluted with ethyl acetate (75 mL). The organic layer was washed with a 1N aqueous hydrochloric acid solution (75 mL) and a saturated aqueous sodium chloride solution (75 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 70-230 mesh, 70% ethyl acetate/hexanes) afforded 2-(3,4-dichloro-phenyl)-3-(3-hydroxyimino-cyclopentyl)-N-thiazol-2-yl-propionamide (138.9 mg, 92%) as a white foam: mp 98-101°C (foam to gel); $(ES)^+$-HRMS m/e calcd for $C_{17}H_{17}Cl_2N_3O_2S$ $(M+H)^+$ 398.0492 found 398.0496.

Example 51
2-(3-Chloro-4-methanesulfonyl-phenyl)-3-(3-hydroxyimino-cyclopentyl)-N-pyrazin-2-yl-propionamide



[0249] A solution of 2-(3-chloro-4-methanesulfonyl-phenyl)-3-(3-oxo-cyclopentyl)-N-pyrazin-2-yl-propionamide (prepared as in Example 45, 121.9 mg, 0.29 mmol) and hydroxylamine hydrochloride (30.4 mg, 0.43 mmol) in methanol (0.85 mL) and pyridine (0.85 mL) was heated under reflux for 5 h. The reaction mixture was allowed to cool to 25°C and was then concentrated *in vacuo*. The resulting residue was diluted with water (50 mL) and extracted with ethyl acetate (50 mL). The organic layer was washed with a 1N aqueous hydrochloric acid solution (2 x 50 mL) and a saturated aqueous sodium chloride solution (50 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 70-230 mesh, 80-100% ethyl acetate/hexanes) afforded 2-(3-chloro-4-methanesulfonyl-phenyl)-3-(3-hydroxyimino-cyclopentyl)-N-pyrazin-2-yl-propionamide (68.8 mg, 55%) as a white solid: mp 205-207°C; $(ES)^+$-HRMS m/e calcd for $C_{19}H_{21}ClN_4O_4S$ $(M+H)^+$ 437.1045 found 437.1048.

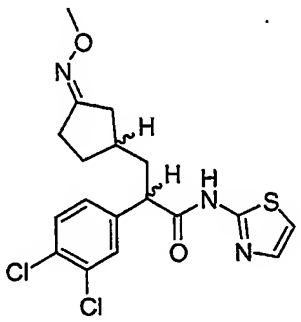
Example 52
N-(5-Bromo-pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(3-hydroxyimino-cyclopentyl)-propionamide



[0250] A solution of N-(5-bromo-pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(3-oxo-cyclopentyl)-propionamide (prepared as in Example 49, 190 mg, 0.38 mmol) and hydroxylamine hydrochloride (40 mg, 0.57 mmol) in methanol (0.90 mL) and pyridine (0.90 mL) was heated under reflux for 3 h. The reaction mixture was allowed to cool to 25°C and was then concentrated *in vacuo*. The resulting residue was diluted with water (50 mL) and extracted with ethyl acetate (50 mL). The organic layers were washed with a 1N aqueous hydrochloric acid solution (50 mL) and a saturated aqueous sodium chloride solution, dried over magnesium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 40S, Silica, 75% ethyl acetate/hexanes) afforded the N-(5-bromo-

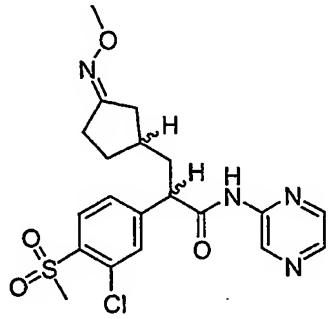
pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(3-hydroxyimino-cyclopentyl)-propionamide (150 mg, 77%) as a yellow foam: mp 100-106°C; $(ES)^+$-HRMS m/e calcd for $C_{19}H_{20}BrClN_4O_4S$ $(M+H)^+$ 515.0150 found 515.0154.

Example 53
2-(3,4-Dichloro-phenyl)-3-(3-methoxyimino-cyclopentyl)-N-thiazol-2-yl-propionamide



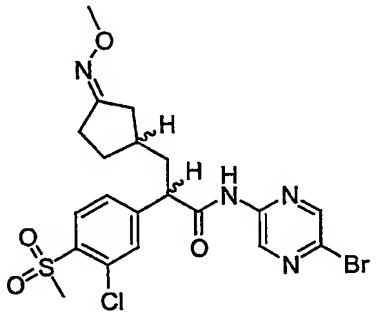
[0251] A solution of the 2-(3,4-dichloro-phenyl)-3-(3-oxo-cyclopentyl)-N-thiazol-2-yl-propionamide (prepared as in Example 43, 159.9 mg, 0.41 mmol) and methoxyamine hydrochloride (52.3 mg, 0.62 mmol) in methanol (1.2 mL) and pyridine (1.2 mL) was heated under reflux for 3.5 h. The reaction mixture was allowed to cool to 25°C and then concentrated *in vacuo*. The resulting residue was diluted with ethyl acetate (75 mL), washed with a 1N aqueous hydrochloric acid solution (75 mL) and a saturated aqueous sodium chloride solution (75 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 50% ethyl acetate/hexanes) afforded 2-(3,4-dichloro-phenyl)-3-(3-methoxyimino-cyclopentyl)-N-thiazol-2-yl-propionamide (168.2 mg, 98%) as a white foam: mp 69-72°C (foam to gel); $(ES)^+$-HRMS m/e calcd for $C_{18}H_{19}Cl_2N_3O_2S$ $(M+H)^+$ 412.0648 found 412.0652.

Example 54
2-(3-Chloro-4-methanesulfonyl-phenyl)-3-(3-methoxyimino-cyclopentyl)-N-pyrazin-2-yl-propionamide



[0252] A solution of 2-(3-chloro-4-methanesulfonyl-phenyl)-3-(3-oxo-cyclopentyl)-N-pyrazin-2-yl-propionamide (prepared as in Example 45, 73.5 mg, 0.17 mmol) and methoxyamine hydrochloride (21.8 mg, 0.26 mmol) in methanol (512 μL) and pyridine (512 μL) was heated under reflux for 5 h. The reaction mixture was allowed to cool to 25°C and was then concentrated *in vacuo*. The resulting residue was diluted with water (50 mL) and extracted with ethyl acetate (2 x 50 mL). The organic layers were washed with a saturated aqueous sodium chloride solution (50 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 70-230 mesh, 70% ethyl acetate/hexanes) afforded 2-(3-chloro-4-methanesulfonyl-phenyl)-3-(3-methoxyimino-cyclopentyl)-N-pyrazin-2-yl-propionamide (61.2 mg, 78%) as a white foam: mp 85-87°C; $(ES)^+$-HRMS m/e calcd for $C_{20}H_{23}ClN_4O_4S$ $(M+H)^+$ 451.1202 found 451.1207.

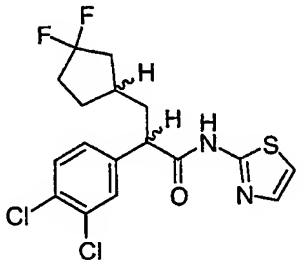
Example 55
N-(5-Bromo-pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(3-methoxyimino-cyclopentyl)-propionamide



[0253] A solution of N-(5-bromo-pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(3-oxo-cyclopentyl)-propionamide (prepared as in Example 49, 190 mg, 0.38 mmol) and methoxyamine hydrochloride (48 mg, 0.57 mmol) in methanol (0.90 mL) and pyridine (0.90 mL) was heated under reflux for 3 h. The reaction mixture was allowed to cool to 25°C and then was concentrated *in vacuo*. The resulting residue was diluted with water (50 mL) and extracted with ethyl acetate (50 mL). The organic layers were washed with a 1N aqueous hydrochloric acid solution (50 mL) and a saturated aqueous sodium chloride solution (50 mL), dried over magnesium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 40S, Silica, 50% ethyl acetate/hexanes) afforded the N-(5-bromo-pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(3-methoxyimino-cyclopentyl)-propionamide (61.2 mg, 78%) as a yellow foam: mp 91-97°C; $(ES)^+$-HRMS m/e calcd for $C_{20}H_{22}BrClN_4O_4S$ $(M+H)^+$ 529.0307 found 529.0310.

Example 56

2-(3,4-Dichloro-phenyl)-3-(3,3-difluoro-cyclopentyl)-N-thiazol-2-yl-propionamide

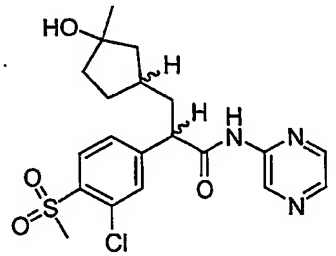


[0254] A mixture of 2-(3,4-dichloro-phenyl)-3-(3-hydroxy-cyclopentyl)-propionic acid methyl ester (prepared as in Example 36, 356.4 mg, 1.12 mmol), *N*-methylmorpholine *N*-oxide (197 mg, 1.68 mmol), and powdered molecular sieves (1.12 g) in methylene chloride (2.25 mL) at 25°C was treated with tetrapropylammonium perrhuthenate (20 mg, 0.05 mmol). The resulting mixture was stirred at 25°C for 30 min. At this time, the reaction was filtered through a pad of celite (ethyl acetate as eluent). The filtrate was concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 75/25 hexanes/ethyl acetate) afforded 2-(3,4-dichloro-phenyl)-3-(3-oxo-cyclopentyl)-propionic acid methyl ester (261.3 mg, 73.8%) as a clear oil: EI-HRMS m/e calcd for $C_{15}H_{16}Cl_2O_3$ $(M+Na)^+$ 337.0370, found 337.0371.

[0255] A solution of 2-(3,4-dichloro-phenyl)-3-(2-oxo-cyclopentyl)-propionic acid methyl ester (261.3 mg, 0.83 mmol) in methylene chloride (0.41 mL) was treated with diethylaminosulfur trifluoride (0.16 mL, 1.24 mmol). The resulting mixture was heated at 60°C for 18 h. At this time, the reaction was cooled to 25°C and was diluted with water (50 mL). This solution was extracted into methylene chloride (3 x 30 mL). The organics were dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 90/10 hexanes/ethyl acetate) afforded 2-(3,4-dichloro-phenyl)-3-(3,3-difluoro-cyclopentyl)-propionic acid methyl ester (79.4 mg, 28.4%) as a yellow oil.

[0256] A mixture of 2-(3,4-dichloro-phenyl)-3-(3,3-difluoro-cyclopentyl)-propionic acid methyl ester (75 mg, 0.22 mmol) and 2-aminothiazole (27 mg, 0.26 mmol) in a solution of magnesium methoxide in methanol (7.4 wt.%, 0.63 mL, 0.44 mmol) was heated to 110°C for 18 h. At this time, the reaction mixture was cooled to 25°C and then concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 230-400 mesh, 70/30 hexanes/ethyl acetate) afforded 2-(3,4-dichloro-phenyl)-3-(3,3-difluoro-cyclopentyl)-N-thiazol-2-yl-propionamide (44.2 mg, 49%) as an off-white solid: mp 154-156°C; FAB-HRMS m/e calcd for $C_{17}H_{16}F_2Cl_2N_2OS$ $(M+H)^+$ 405.0402, found 405.0404.

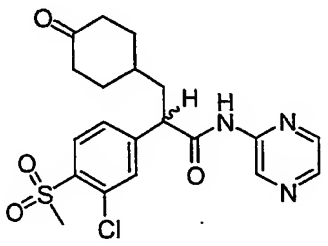
Example 57
2-(3-Chloro-4-methanesulfonyl-phenyl)-3-(3-hydroxy-3-methyl-cyclopentyl)-N-pyrazin-2-yl-propionamide



[0257] A 3.0M solution of methylmagnesium bromide in ether (2.53 mL, 7.59 mmol) cooled to 0°C was treated dropwise with a solution of 2-(3-chloro-4-methanesulfonyl-phenyl)-3-(3-oxo-cyclopentyl)-N-pyrazin-2-yl-propionamide

(prepared as in Example 45, 0.10 g, 0.237 mmol) in tetrahydrofuran (1 mL). The resulting solution was stirred at 0°C for 30 min, quenched with a saturated aqueous ammonium chloride solution (3 mL), and partitioned between a saturated aqueous ammonium chloride solution (20 mL) and ethyl acetate (25 mL). The aqueous layer was extracted with ethyl acetate (25 mL). The combined organic layers were dried over magnesium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 40S, Silica, 80% ethyl acetate/hexanes) afforded 2-(3-chloro-4-methanesulfonyl-phenyl)-3-(3-hydroxy-3-methyl-cyclopentyl)-N-pyrazin-2-yl-propionamide (37 mg, 35%) as a yellow foam: mp 78-84°C (foam to gel); $(ES)^+$-HRMS m/e calcd for $C_{20}H_{24}ClN_3O_4S$ $(M+H)^+$ 438.1249 found 438.1254.

Example 58
2-(3-Chloro-4-methanesulfonyl-phenyl)-3-(4-oxo-cyclohexyl)-N-pyrazin-2-yl-propionamide



[0258] A solution of 4-cyclohexanonecarboxylic acid ethyl ester (5.00 g, 29.38 mmol) and ethylene glycol (2.13 mL, 38.19 mmol) in toluene (150 mL) was heated in a Dean-Stark trap at reflux for 1 h and was then treated with *p*-toluenesulfonic acid monohydrate (56.74 mg, 0.294 mmol). The reaction solution was heated an additional 30 min at reflux, cooled to 25°C, and concentrated *in vacuo*. The resulting oil was dissolved in ethyl acetate (200 mL), washed with water (2 x 50 mL), dried over magnesium sulfate, filtered, and concentrated *in vacuo* to afford pure 1,4-dioxa-spiro[4.5]decane-8-carboxylic acid ethyl ester (6.25 g, 99.3%) as a colorless oil.

[0259] A solution of 1,4-dioxa-spiro[4.5]decane-8-carboxylic acid ethyl ester (2.00 g, 9.33 mmol) in tetrahydrofuran (40 mL) at 0°C was treated dropwise with a 1.0M

solution of lithium aluminum hydride in tetrahydrofuran (10.0 mL, 10.0 mmol). The reaction mixture was stirred at 0°C for 30 min and was then quenched by the dropwise addition of ethyl acetate. The reaction was then diluted with a saturated aqueous ammonium chloride solution (25 mL) and extracted with ethyl acetate (3 x 25 mL). The combined organic extracts were dried over sodium sulfate, filtered, and concentrated *in vacuo* to afford pure (1,4-dioxa-spiro[4.5]dec-8-yl)-methanol (1.60 g, 99.9%) as a colorless oil.

[0260] A solution of (1,4-dioxa-spiro[4.5]dec-8-yl)-methanol (1.60 g, 9.29 mmol) in methylene chloride (50 mL) was treated with 4-(dimethylamino)pyridine (1.26 g, 10.22 mmol) and *p*-toluenesulfonyl chloride (1.86 g, 9.75 mmol). The reaction mixture was stirred at 25°C for 2 h, and was then washed with a saturated aqueous sodium bicarbonate solution (1 x 25 mL). The organic layer was dried over magnesium sulfate, filtered, and concentrated *in vacuo*. The resulting oil was dissolved in acetone (30 mL) and treated with sodium iodide (4.73 g, 31.53 mmol). The reaction was heated under reflux for 2 h, cooled to 25°C, and then concentrated *in vacuo*. The resulting residue was suspended in ethyl acetate (50 mL), washed with water (2 x 15 mL), dried over magnesium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 40S, Silica, 19/1 hexanes/ethyl acetate) afforded 8-iodomethyl-1,4-dioxa-spiro[4.5]decane (2.32 g, 88.6%) as a colorless oil.

[0261] A solution of diisopropylamine (0.23 mL, 1.63 mmol) in dry tetrahydrofuran (5.0 mL) cooled to -78°C under nitrogen was treated with a 2.5M solution of *n*-butyllithium in hexanes (0.65 mL, 1.63 mmol). The reaction mixture was stirred at -78°C for 30 min and then treated dropwise with a solution of (3-chloro-4-methylsulfanyl-phenyl)-acetic acid methyl ester (prepared as in Example 4, 340 mg, 1.48 mmol) in dry tetrahydrofuran (3.0 mL) and 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (1.0 mL). The reaction mixture turned dark in color and was allowed to stir at -78°C for 1 h, at which time, a solution of 8-iodomethyl-1,4-dioxa-spiro[4.5]decane (500 mg, 1.78 mmol) in a small amount of dry tetrahydrofuran was added dropwise. The reaction mixture was allowed to warm to 25°C, where it was stirred for 24 h. The reaction mixture was quenched

with a saturated aqueous ammonium chloride solution and then concentrated *in vacuo* to remove tetrahydrofuran. The aqueous residue was acidified using a 10% aqueous hydrochloric acid solution. The resulting aqueous layer was extracted with ethyl acetate (2 x 100 mL). The combined organic extracts were dried over sodium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 40S Silica 8/2 hexanes/ethyl acetate) afforded the 2-(3-chloro-4-methylsulfanyl-phenyl)-3-(1,4-dioxa-spiro[4.5]dec-8-yl)-propionic acid methyl ester (315 mg, 55%) as a yellow viscous oil: EI-HRMS m/e calcd for $C_{19}H_{25}ClO_4S$ (M^+) 384.1162, found 384.1169.

[0262] A solution of 2-(3-chloro-4-methylsulfanyl-phenyl)-3-(1,4-dioxa-spiro[4.5]dec-8-yl)-propionic acid methyl ester (746 mg, 1.93 mmol) in methylene chloride (22 mL) cooled to 0°C was treated dropwise with a pre-mixed solution of 3-chloroperoxybenzoic acid (70% grade, 955 mg, 3.86 mmol) and sodium bicarbonate (652 mg, 7.72 mmol) in methylene chloride (11 mL). The reaction mixture was stirred at 25°C for 4.5 h, after which time, the reaction mixture was diluted with methylene chloride (100 mL) and washed with water (100 mL). The organic phase was cooled to 0°C and successively washed with a 10% aqueous sodium sulfite solution (100 mL), a saturated aqueous sodium bicarbonate solution (100 mL), and a 50% aqueous sodium chloride solution (100 mL). The combined organic extracts were dried over sodium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 40S Silica, 6/4 hexanes/ethyl acetate) afforded 2-(3-chloro-4-methylsulfonyl-phenyl)-3-(1,4-dioxa-spiro[4.5]dec-8-yl)-propionic acid methyl ester (680 mg, 84%) as a colorless gum: EI-HRMS m/e calcd for $C_{19}H_{25}ClO6_4S$ $(M+Na)^+$ 439.0953, found 439.0957.

[0263] A solution of 2-(3-chloro-4-methylsulfonyl-phenyl)-3-(1,4-dioxa-spiro[4.5]dec-8-yl)-propionic acid methyl ester (667 mg, 1.60 mmol) in acetone (15 mL) was treated with a 10% aqueous hydrochloric acid solution (1.8 mL). The reaction mixture was stirred at 25°C for 24 h. The reaction mixture was then diluted with water (50 mL) and extracted with ethyl acetate (100 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated *in vacuo* to afford pure 2-(3-chloro-4-methylsulfonyl-phenyl)-3-(4-oxo-cyclohexyl)-propionic acid

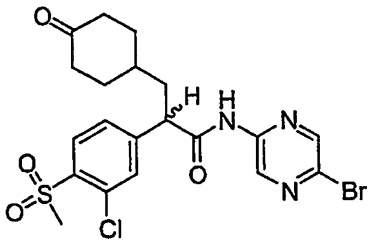
methyl ester (600 mg, 100%) as a colorless gum which was used without further purification: EI-HRMS m/e calcd for $C_{17}H_{21}ClO_5S$ $(M+Na)^+$ 395.0690, found 395.0692.

[0264] A solution of 2-(3-chloro-4-methylsulfonyl-phenyl)-3-(4-oxo-cyclohexyl)-propionic acid methyl ester (590 mg, 1.58 mmol) in methanol (7.0 mL) and water (3.0 mL) was treated with lithium hydroxide (980 mg, 31.6 mmol). The reaction mixture was stirred at 25°C for 2 h and then concentrated *in vacuo* to remove methanol. The remaining aqueous layer was diluted with water (25 mL) and washed with ethyl acetate (2 x 50 mL). The aqueous layer was then acidified to pH=3 with a 1N aqueous hydrochloric acid solution and was extracted with ethyl acetate (2 x 50 mL). The combined organic layers were washed with a saturated aqueous sodium chloride solution (1 x 50 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo* to afford pure 2-(3-chloro-4-methylsulfonyl-phenyl)-3-(4-oxo-cyclohexyl)-propionic acid (550 mg, 97%) as a colorless gum: EI-HRMS m/e calcd for $C_{16}H_{19}ClO_5S$ $(M+Na)^+$ 381.0534, found 381.0537.

[0265] A solution of 2-(3-chloro-4-methylsulfonyl-phenyl)-3-(4-oxo-cyclohexyl)-propionic acid (267 mg, 0.75 mmol) in methylene chloride (10 mL) was treated with *N,N*-dimethylformamide (3 drops) and then cooled to 0°C. The reaction mixture was then treated with a 2.0M solution of oxalyl chloride in methylene chloride (0.45 mL, 0.90 mmol). The reaction mixture was stirred at 25°C for 30 min and then concentrated *in vacuo* to remove solvents and excess oxalyl chloride. The resulting residue was re-dissolved in dry tetrahydrofuran (10 mL) and was treated dropwise with a solution of 2-aminopyrazine (143 mg, 1.50 mmol) in tetrahydrofuran (10 mL) and pyridine (0.30 mL, 3.75 mmol). The resulting reaction mixture was stirred at 25°C for 2 h. The reaction mixture was then diluted with a 1N aqueous citric acid solution (25 mL) and concentrated to remove tetrahydrofuran. The remaining aqueous residue was then extracted with a 3/2 solution of chloroform/methanol (2 x 25 mL). The combined organic extracts were dried over sodium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 40S, Silica, 3/2 hexanes/ethyl acetate) afforded 2-(3-chloro-4-methanesulfonyl-phenyl)-3-(4-oxo-cyclohexyl)-N-pyrazin-2-yl-

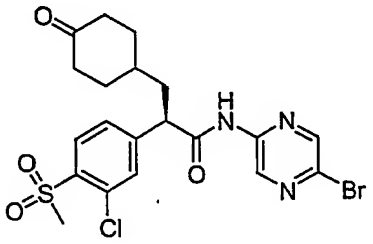
propionamide (266 mg, 81%) as an off-white foam: EI-HRMS m/e calcd for $C_{20}H_{22}ClN_3O_4S$ $(M+H)^+$ 436.1093, found 436.1099.

Example 59
N-(5-Bromo-pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(4-oxo-cyclohexyl)-propionamide



[0266] A solution of 2-(3-chloro-4-methylsulfonyl-phenyl)-3-(4-oxo-cyclohexyl)-propionic acid (prepared as in Example 58, 267 mg, 0.75 mmol) in methylene chloride (10 mL) was treated with *N,N*-dimethylformamide (3 drops) and then cooled to 0°C. The reaction mixture was then treated with a 2.0M solution of oxalyl chloride in methylene chloride (0.45 mL, 0.90 mmol). The reaction mixture was stirred at 25°C for 30 min and then concentrated *in vacuo* to remove solvents and excess oxalyl chloride. The resulting residue was re-dissolved in dry tetrahydrofuran (10 mL) and was treated dropwise with a solution of 2-amino-5-bromopyrazine (261 mg, 1.50 mmol, prepared according to *Tetrahedron* **1988**, *44*, 2977-2983) in tetrahydrofuran (10 mL) and pyridine (0.30 mL, 3.75 mmol). The resulting reaction mixture was stirred at 25°C for 2 h. The reaction mixture was then diluted with a 1N aqueous citric acid solution (25 mL) and then concentrated to remove tetrahydrofuran. The remaining aqueous residue was then extracted with a 3/2 solution of chloroform/methanol (2 x 25 mL). The combined organic extracts were dried over sodium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 40M, Silica, 35/65 hexanes/ethyl acetate) afforded N-(5-bromo-pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(4-oxo-cyclohexyl)-propionamide (193 mg, 50%) as a yellow foam: EI-HRMS m/e calcd for $C_{20}H_{21}BrClN_3O_4S$ $(M+H)^+$ 514.0198, found 514.0200.

Example 60
N-(5-Bromo-pyrazin-2-yl)-2(R)-(3-chloro-4-methanesulfonyl-phenyl)-3-(4-oxo-cyclohexyl)-propionamide



[0267] A mixture of (3-chloro-4-methylsulfanyl-phenyl)-acetic acid (prepared as in Example 4, 10.48 g, 48.4 mmol) and potassium carbonate (20.1 g, 145.1 mmol) in acetone (65 mL) was cooled to -10°C. The pale yellow slurry was then treated dropwise with trimethylacetyl chloride (6.25 mL, 50.8 mmol) while maintaining the temperature below -10°C. The resulting reaction mixture was stirred at -10°C for 15 min and then allowed to warm to 0°C, where it was stirred for 10 min. The reaction mixture was re-cooled to -10°C and then treated with (1R, 2R)-(-)-pseudoephedrine (11.99 g, 72.5 mmol), resulting in an exotherm. The reaction mixture was stirred at -10°C for 10 min and then warmed to 25°C, where it was stirred for 1 h. At this time, the reaction mixture was then quenched with water (50 mL) and then extracted with ethyl acetate (1 x 100 mL). The organic layer was washed with water (2 x 40 mL). The aqueous layers were combined and back-extracted with ethyl acetate (2 x 50 mL). The combined organic layers were dried over magnesium sulfate, filtered, and concentrated *in vacuo*. The crude material was recrystallized from ethyl acetate (45 mL) and hexanes (80 mL) to afford 2-(3-chloro-4-methylsulfanyl-phenyl)-N-[2(R)-hydroxy-1(R)-methyl-2(R)-phenyl-ethyl]-N-methyl-acetamide (13.75 g, 78%) as a light yellow solid: mp 111.5-112.9°C; $[\alpha]^{23}_{589}$ = –97.2° (c=0.104, chloroform); FAB-HRMS m/e calcd for $C_{19}H_{22}ClNSO_2$ $(M+H)^+$ 364.1138, found 364.1142.

[0268] A solution of 1,1,1,3,3,3-hexamethyldisilazane (9.73 mL, 46.07 mmol) in tetrahydrofuran (35 mL) cooled to -78°C was treated with a 2.5M solution of *n*-butyllithium in hexanes (18.00 mL, 45.0 mmol). The reaction mixture was stirred at -78°C for 15 min and then slowly treated with a solution of 2-(3-chloro-4-methylsulfanyl-phenyl)-N-[2(R)-hydroxy-1(R)-methyl-2(R)-phenyl-ethyl]-N-

methyl-acetamide (6.45 g, 17.72 mmol) in tetrahydrofuran (35 mL) while maintaining the temperature below -65°C. The resulting yellow-orange reaction mixture was stirred at -78°C for 15 min and then allowed to warm to 0°C, where it was stirred for 20 min. The reaction mixture was then re-cooled to -78°C and then treated with a solution of 8-iodomethyl-1,4-dioxa-spiro[4.5]decane (prepared as in Example 58, 10.00 g, 35.4 mmol) in 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (3.0 mL) and tetrahydrofuran (10 mL) The resulting reaction mixture was stirred at -78°C for 30 min and then allowed to warm to 25°C, where it was stirred for 16 h. The reaction mixture was diluted with ethyl acetate (100 mL) and then was washed with a saturated aqueous ammonium chloride solution (1 x 50 mL). The aqueous layer was then extracted with ethyl acetate (2 x 50 mL). The combined organic layers were washed with a saturated aqueous sodium chloride solution (1 x 50 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo*. The resulting material was then re-dissolved in ethyl acetate. This organic phase was washed with a 10% aqueous sulfuric acid solution (2 x 100 mL) and a 10% aqueous sodium bicarbonate solution (2 x 100 mL), dried over magnesium sulfate, filtered, and concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 70-230 mesh, 2/3 hexanes/ethyl acetate to 1/4 hexanes/ethyl acetate) afforded the 2(R)-(3-chloro-4-methylsulfanyl-phenyl)-3-(1,4-dioxa-spiro[4.5]dec-8-yl)-N-(2(R)-hydroxy-1(R)-methyl-2(R)-phenyl-ethyl)-N-methyl-propionamide (9.11 g, 99.0%) as a white foam: $[\alpha]^{23}_{589} = -79.49^{\circ}$ (c=0.39, chloroform); EI-HRMS m/e calcd for $C_{28}H_{36}ClNO_4S$ $(M+H)^+$ 518.2127, found 518.2123.

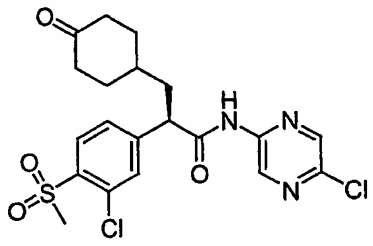
[0269] A solution of 2(R)-(3-chloro-4-methylsulfanyl-phenyl)-3-(1,4-dioxa-spiro[4.5]dec-8-yl)-N-(2(R)-hydroxy-1(R)-methyl-2(R)-phenyl-ethyl)-N-methyl-propionamide (6.30 g, 12.16 mmol) in methylene chloride (70 mL) cooled to 0°C was treated dropwise with a pre-mixed solution of 3-chloroperoxybenzoic acid (70%, 6.00 g, 24.32 mmol) and sodium bicarbonate (4.09 g, 48.64 mmol) in methylene chloride (70 mL) over a 10 min period. The resulting mixture was allowed to warm to 25°C, where it was stirred for 2 h. The reaction mixture was diluted with methylene chloride (100 mL), re-cooled to 0°C, and treated dropwise with an additional solution of 3-chloroperoxybenzoic acid (70%, 3.00 g, 12.16

mmol) and sodium bicarbonate (2.05 g, 24.32 mmol) in methylene chloride (35 mL). The resulting mixture was allowed to warm to 25°C, where it was stirred for 2 h. The reaction mixture was then diluted with methylene chloride (1 L), washed with water (1 x 500 mL), cooled to 0°C, and then washed sequentially with a saturated aqueous sodium bisulfite solution (1 x 500 mL), a saturated aqueous sodium bicarbonate solution (1 x 500 mL), and a saturated aqueous sodium chloride solution (1 x 500 mL). The combined organic extracts were dried over magnesium sulfate and concentrated *in vacuo*. The resulting white foam was then dissolved in dioxane (40 mL), treated with a 9N aqueous sulfuric acid solution (40 mL), and heated at 105°C for 16 h. The reaction mixture was then cooled to 0°C with an ice bath, and the product was precipitated by adding water (500 mL). The suspension was stirred at 0°C until the supernatant, which was initially turbid, became clear and light yellow in color. The solid was filtered off and dried by suction. The solid material was dissolved in hot glacial acetic acid (40 mL), and the hot solution was treated with water (25 mL) to initiate crystallization. The mixture was allowed to cool to 25°C and then treated with an additional amount of water (50 mL). After stirring at 25°C for 1 h, the solid was collected by filtration. The solid was dried in a high vacuum desiccator with phosphorus pentoxide to afford 2(R)-(3-chloro-4-methanesulfonyl-phenyl)-3-(4-oxo-cyclohexyl)-propionic acid (4.10 g, 94%) as an off-white solid: mp 129-131°C; $[\alpha]^{23}_{589}$ = –42.22° (c=0.36, chloroform); EI-HRMS m/e calcd for $C_{16}H_{19}ClO_5S$ $(M+Na)^+$ 381.0534, found 381.0536.

[0270] A solution of 2(R)-(3-chloro-4-methanesulfonyl-phenyl)-3-(4-oxo-cyclohexyl)-propionic acid (200 mg, 0.56 mmol) and triphenylphosphine (195 mg, 0.73 mmol) in methylene chloride (4.0 mL) cooled to 0°C was treated with *N*-bromosuccinimide (128 mg, 0.73 mmol) in small portions. After the complete addition of *N*-bromosuccinimide, the reaction mixture was allowed to warm to 25°C over 30 min. The bright orange reaction mixture was then treated with 2-amino-5-bromopyrazine (200 mg, 1.12 mmol, prepared according to *Tetrahedron* **1988**, *44*, 2977-2983) and 2,6-lutidine (0.28 mL, 2.24 mmol). The resulting reaction mixture was stirred at 25°C for 4 h. The reaction mixture was then diluted with methylene chloride (25 mL) and was successively washed with a 10%

aqueous hydrochloric acid solution (1 x 20 mL), a saturated aqueous sodium bicarbonate solution (1 x 20 mL) and water (1 x 20 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 40S, Silica, 65/35 hexanes/ethyl acetate eluted to 4/6 hexanes/ethyl acetate) afforded N-(5-bromo-pyrazin-2-yl)-2(R)-(3-chloro-4-methanesulfonyl-phenyl)-3-(4-oxo-cyclohexyl)-propionamide (120 mg, 42%) as an off-white foam: $[\alpha]^{23}_{589}$ = –24.6° (c=0.50, chloroform); EI-HRMS m/e calcd for $C_{20}H_{21}BrClN_3O_4S$ $(M+Na)^+$ 536.0017, found 536.0022.

Example 61
2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-N-(5-chloro-pyrazin-2-yl)-3-(4-oxo-cyclohexyl)-propionamide

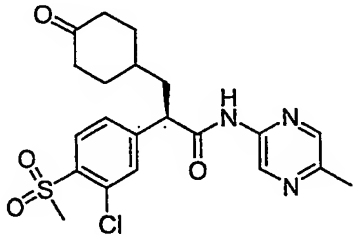


[0271] A solution of 2-aminopyrazine (23.86 g, 0.2509 mol) in methylene chloride (420 mL) was cooled to 0°C and then treated with *N*-chlorosuccinimide (33.50 g, 0.2509 mol). The reaction mixture was stirred at 0°C for 24 h. The resulting dark reaction mixture was diluted with water (500 mL) and then concentrated *in vacuo* to remove methylene chloride. The aqueous layer was continuously extracted with ethyl acetate until product was absence from the aqueous layer as determined by thin layer chromatography. The combined organic layers were dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash chromatography (Merck Silica gel 60, 70-230 mesh, 25% ethyl acetate/hexanes) afforded 2-amino-5-chloropyrazine (2.66 g, 8.2%) as a yellow solid: mp 126-128°C; EI-HRMS m/e calcd for $C_4H_4ClN_3$ (M^+) 129.0094, found 129.0090.

[0272] A solution of 2(R)-(3-chloro-4-methanesulfonyl-phenyl)-3-(4-oxo-cyclohexyl)-propionic acid (prepared as in Example 60, 200 mg, 0.56 mmol) and triphenylphosphine (192 mg, 0.73 mmol) in methylene chloride (4.0 mL) cooled to 0°C was treated with *N*-bromosuccinimide (128 mg, 0.73 mmol) in small portions.

After the complete addition of *N*-bromosuccinimide, the reaction mixture was allowed to warm to 25°C over 30 min. The bright orange reaction mixture was then treated with 2-amino-5-chloropyrazine (145 mg, 1.12 mmol) and 2,6-lutidine (0.28 mL, 2.24 mmol). The resulting reaction mixture was stirred at 25°C for 4 h. The reaction mixture was then diluted with methylene chloride (25 mL) and was successively washed with a 10% aqueous hydrochloric acid solution (1 x 20 mL), a saturated aqueous sodium bicarbonate solution (1 x 20 mL) and water (1 x 20 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 40S, Silica, 13/7 hexanes/ethyl acetate to 2/3 hexanes/ethyl acetate) afforded 2(R)-(3-chloro-4-methanesulfonyl-phenyl)-N-(5-chloro-pyrazin-2-yl)-3-(4-oxo-cyclohexyl)-propionamide (137 mg, 52%) as a light yellow foam: $[\alpha]^{23}_{589} = -27.35°$ (c=0.49, chloroform); EI-HRMS m/e calcd for $C_{20}H_{21}Cl_2N_3O_4S$ $(M+H)^+$ 470.0703, found 470.0705.

Example 62
2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-N-(5-methyl-pyrazin-2-yl)-3-(4-oxo-cyclohexyl)-propionamide



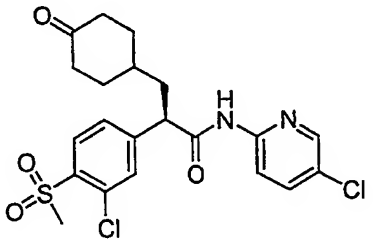
[0273] A mixture of 5-methylpyrazine-2-carboxylic acid (2.76 g, 20 mmol) and oxalyl chloride (1.83 mL, 2.66 g, 21 mmol) in methylene chloride (40mL) was treated with *N,N*-dimethylformamide (0.5 mL), and the mixture was stirred at 25°C for 1 h. The mixture was filtered, and the filtrate was concentrated *in vacuo* to give an oily solid. The solid was dissolved in acetone (120 mL) at 0°C and then sodium azide (1.03g, 20 mmol) in water (50 mL) was added dropwise. After the addition was complete, stirring was continued at 0°C for 30 min. The mixture was then poured into ice cold water (100 mL) and extracted with methylene chloride (3 x 100 mL). The combined organic extracts were washed with water (1 x 100 mL), a mixture of a saturated aqueous sodium bicarbonate solution and a saturated

aqueous sodium chloride solution (1:1, 1 x 100 mL), and dried over anhydrous sodium sulfate. The mixture was filtered and concentrated *in vacuo* to give 5-methyl-pyrazine-2-carbonyl azide (1.46 g, 45%) as a tan solid. The 5-methyl-pyrazine-2-carbonyl azide (500 mg, 3.07 mmol) was combined with benzyl alcohol (0.63 mL, 663 mg, 6.14 mmol) at 25°C. The mixture was then slowly heated on an oil bath to 90°C, upon which gas was violently evolved. The oil bath temperature was maintained until gas evolution ceased. The oil bath temperature was raised to 120°C and stirring was continued for 10 min at that temperature. The mixture was cooled and triturated with diethyl ether/hexanes (1:4) to give (5-methylpyrazin-2-yl)-carbamic acid phenyl ester (438 mg, 58%) as a yellow solid. The (5-methylpyrazin-2-yl)-carbamic acid phenyl ester (500 mg, 2.2 mmol) and 10% palladium on carbon (212 mg) were mixed in ethanol (30 mL). The reaction vessel was flushed with hydrogen, and the mixture was stirred at 25°C for 1 h under hydrogen (1 atm). The excess hydrogen was evacuated from the reaction vessel, and the mixture was filtered through a pad of celite. Concentration of the filtrate *in vacuo* gave 2-amino-5-methylpyrazine (183 mg , 76%) as a tan solid which was used without further purification.

[0274] A solution of 2(R)-(3-chloro-4-methanesulfonyl-phenyl)-3-(4-oxo-cyclohexyl)-propionic acid (prepared as in Example 60, 263 mg, 0.73 mmol) and triphenylphosphine (250 mg, 0.95 mmol) in methylene chloride (5.0 mL) cooled to 0°C was treated with *N*-bromosuccinimide (167 mg, 0.95 mmol) in small portions. After the complete addition of *N*-bromosuccinimide, the reaction mixture was allowed to warm to 25°C over 30 min. The bright orange reaction mixture was then treated with 2-amino-5-methylpyrazine (160 mg, 1.46 mmol) and 2,6-lutidine (0.36 mL, 2.92 mmol). The resulting reaction mixture was stirred at 25°C for 4 h. The reaction mixture was then diluted with methylene chloride (25 mL) and was successively washed with a 10% aqueous hydrochloric acid solution (1 x 20 mL), a saturated aqueous sodium bicarbonate solution (1 x 20 mL) and water (1 x 20 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 40S, Silica, 65/35 hexanes/ethyl acetate to 3/7 hexanes/ethyl acetate) afforded 2(R)-(3-chloro-4-methanesulfonyl-phenyl)-N-(5-methyl-pyrazin-2-yl)-3-(4-oxo- cyclohexyl)-propionamide (158 mg, 48%) as a

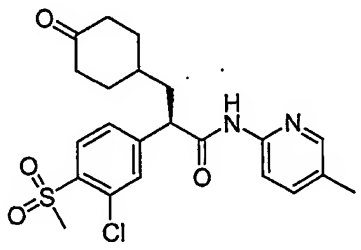
white foam: $[\alpha]^{23}_{589}$ = –41.52° (c=0.33, chloroform); EI-HRMS m/e calcd for $C_{20}H_{21}Cl_2N_3O_4S$ $(M+H)^+$ 450.1249, found 450.1253.

Example 63
2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-N-(5-chloro-pyridin-2-yl)-3-(4-oxo-cyclohexyl)-propionamide



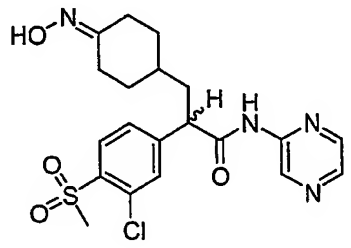
[0275] A solution of 2(R)-(3-chloro-4-methanesulfonyl-phenyl)-3-(4-oxo-cyclohexyl)-propionic acid (prepared as in Example 60, 300 mg, 0.84 mmol) and triphenylphosphine (288 mg, 1.09 mmol) in methylene chloride (6.0 mL) cooled to 0°C was treated with *N*-bromosuccinimide (192 mg, 1.09 mmol) in small portions. After the complete addition of *N*-bromosuccinimide, the reaction mixture was allowed to warm to 25°C over 30 min. The bright orange reaction mixture was then treated with 2-amino-5-chloropyridine (220 mg, 1.68 mmol) and 2,6-lutidine (0.42 mL, 3.36 mmol). The resulting reaction mixture was stirred at 25°C for 4 h. The reaction mixture was then diluted with methylene chloride (25 mL) and was successively washed with a 10% aqueous hydrochloric acid solution (1 x 20 mL), a saturated aqueous sodium bicarbonate solution (1 x 20 mL) and water (1 x 20 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 40S, Silica, 65/35 hexanes/ethyl acetate to 1/1 hexanes/ethyl acetate) afforded 2(R)-(3-chloro-4-methanesulfonyl-phenyl)-N-(5-chloro-pyridin-2-yl)-3-(4-oxo- cyclohexyl)-propionamide (265mg, 67%) as a white foam: $[\alpha]^{23}_{589}$ = –35.71° (c=0.35, chloroform); EI-HRMS m/e calcd for $C_{21}H_{22}Cl_2N_2O_4S$ $(M+H)^+$ 469.0750, found 469.0754.

Example 64
2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-N-(5-methyl-pyridin-2-yl)-3-(4-oxo-cyclohexyl)-propionamide



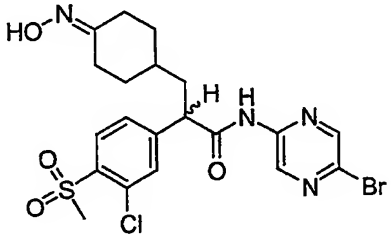
[0276] A solution of 2(R)-(3-chloro-4-methanesulfonyl-phenyl)-3-(4-oxo-cyclohexyl)-propionic acid (prepared as in Example 60, 300 mg, 0.84 mmol) and triphenylphosphine (288 mg, 1.09 mmol) in methylene chloride (6.0 mL) cooled to 0°C was treated with *N*-bromosuccinimide (192 mg, 1.09 mmol) in small portions. After the complete addition of *N*-bromosuccinimide, the reaction mixture was allowed to warm to 25°C over 30 min. The bright orange reaction mixture was then treated with 2-amino-5-picoline (182 mg, 1.68 mmol) and 2,6-lutidine (0.42 mL, 3.36 mmol). The resulting reaction mixture was stirred at 25°C for 4 h. The reaction mixture was then diluted with methylene chloride (25 mL) and was successively washed with a 10% aqueous hydrochloric acid solution (1 x 20 mL), a saturated aqueous sodium bicarbonate solution (1 x 20 mL) and water (1 x 20 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 40S, Silica, 65/35 hexanes/ethyl acetate to 4/6 hexanes/ethyl acetate) afforded 2(R)-(3-chloro-4-methanesulfonyl-phenyl)-N-(5-methyl-pyridin-2-yl)-3-(4-oxo-cyclohexyl)-propionamide (231 mg, 61%) as a white foam: $[\alpha]^{23}_{589} = -26.22°$ (c=0.45, chloroform); EI-HRMS m/e calcd for $C_{22}H_{25}ClN_2O_4S$ $(M+H)^+$ 449.1297, found 449.1302.

Example 65
2-(3-Chloro-4-methanesulfonyl-phenyl)-3-(4-hydroxyimino-cyclohexyl)-N-pyrazin-2-yl-propionamide



[0277] A solution of hydroxylamine hydrochloride (19 mg, 0.27 mmol) in methanol (0.5 mL) and pyridine (0.5 mL) was treated with 2-(3-chloro-4-methanesulfonyl-phenyl)-3-(4-oxo-cyclohexyl)-N-pyrazin-2-yl-propionamide (prepared as in Example 58, 80 mg, 0.18 mmol). The reaction mixture was heated under reflux for 2 h, cooled to 25°C, and concentrated *in vacuo* to remove methanol. The resulting residue was suspended in ethyl acetate (10 mL), washed with water (1 x 5 mL), dried over magnesium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 12M, Silica, 35/65 hexanes/ethyl acetate to 1/1 hexanes/ethyl acetate) afforded 2-(3-chloro-4-methanesulfonyl-phenyl)-3-(4-hydroxyimino-cyclohexyl)-N-pyrazin-2-yl-propionamide (65 mg, 80%) was a white foam: EI-HRMS m/e calcd for $C_{20}H_{23}ClN_4O_4S$ $(M+H)^+$ 451.1202, found 451.1206.

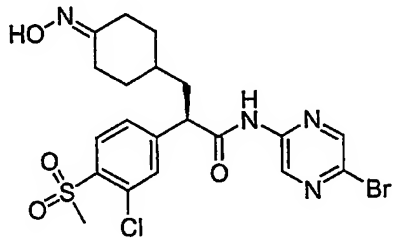
Example 66
N-(5-Bromo-pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(4-hydroxyimino-cyclohexyl)-propionamide



[0278] A solution of hydroxylamine hydrochloride (13 mg, 0.18 mmol) in methanol (0.5 mL) and pyridine (0.5 mL) was treated with N-(5-bromo-pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(4-oxo-cyclohexyl)-propionamide (prepared as in Example 59, 62 mg, 0.12 mmol). The reaction mixture was heated under

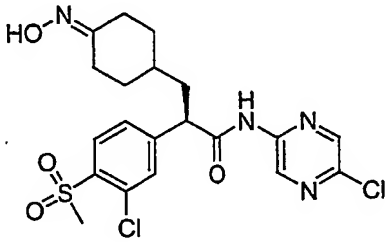
reflux for 2 h, cooled to 25°C, and concentrated *in vacuo* to remove methanol. The resulting residue was suspended in ethyl acetate (10 mL), washed with water (1 x 5 mL), dried over magnesium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 12M, Silica, 3/7 hexanes/ethyl acetate eluted to 8/2 hexanes/ethyl acetate) afforded the N-(5-bromo-pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(4-hydroxyimino-cyclohexyl)-propionamide (51 mg, 80%) as a white foam: EI-HRMS m/e calcd for $C_{20}H_{22}BrClN_4O_4S$ $(M+H)^+$ 529.0307, found 529.0308.

Example 67
N-(5-Bromo-pyrazin-2-yl)-2(R)-(3-chloro-4-methanesulfonyl-phenyl)-3-(4-hydroxyimino-cyclohexyl)-propionamide



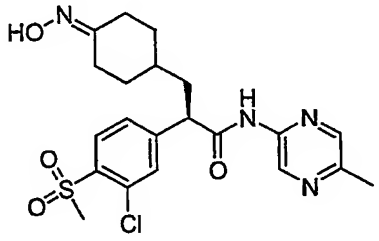
[0279] A solution of hydroxylamine hydrochloride (7.0 mg, 0.099 mmol) in methanol (0.2 mL) and 2,6-lutidine (0.2 mL) was treated with N-(5-bromo-pyrazin-2-yl)-2(R)-(3-chloro-4-methanesulfonyl-phenyl)-3-(4-oxo-cyclohexyl)-propionamide (prepared as in Example 60, 34 mg, 0.066 mmol). The reaction mixture was stirred at 25°C for 30 min and was then concentrated *in vacuo* to remove methanol. The resulting residue was suspended in ethyl acetate (10 mL), washed with water (1 x 5 mL), dried over magnesium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 12M, Silica, 65/35 hexanes/ethyl acetate to 2/3 hexanes/ethyl acetate) afforded the N-(5-bromo-pyrazin-2-yl)-2(R)-(3-chloro-4-methanesulfonyl-phenyl)-3-(4-hydroxyimino-cyclohexyl)-propionamide (30 mg, 85.8%) was a white foam: $[\alpha]^{23}_{589} = -21.43°$ (c=0.35, chloroform); EI-HRMS m/e calcd for $C_{20}H_{22}BrClN_4O_4S$ $(M+H)^+$ 529.0307, found 529.0314.

Example 68
2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-N-(5-chloro-pyrazin-2-yl)-3-(4-hydroxyimino-cyclohexyl)-propionamide



[0280] A solution of hydroxylamine hydrochloride (7.0 mg, 0.099 mmol) in methanol (0.2 mL) and 2,6-lutidine (0.2 mL) was treated with 2(R)-(3-chloro-4-methanesulfonyl-phenyl)-N-(5-chloro-pyrazin-2-yl)-3-(4-oxo-cyclohexyl)-propionamide (prepared as in Example 61, 31 mg, 0.066 mmol). The reaction mixture was stirred at 25°C for 30 min and was then concentrated *in vacuo* to remove methanol. The resulting residue was suspended in ethyl acetate (10 mL), washed with water (1 x 5 mL), dried over magnesium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 12S, Silica, 13/7 hexanes/ethyl acetate to 2/3 hexanes/ethyl acetate) afforded 2(R)-(3-chloro-4-methanesulfonyl-phenyl)-N-(5-chloro-pyrazin-2-yl)-3-(4-hydroxyimino-cyclohexyl)-propionamide (30 mg, 93.7%) was a white foam: $[\alpha]^{23}_{589}$ = –23.24° (c=0.37, chloroform); EI-HRMS m/e calcd for $C_{20}H_{22}Cl_2N_4O_4S$ $(M+H)^+$ 485.0812, found 485.0822.

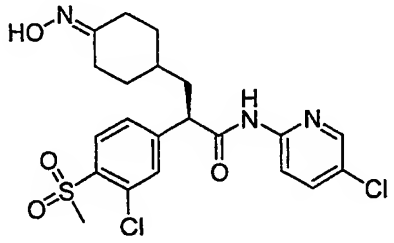
Example 69
2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-3-(4-hydroxyimino-cyclohexyl)-N-(5-methyl-pyrazin-2-yl)-propionamide



[0281] A solution of hydroxylamine hydrochloride (17.0 mg, 0.24 mmol) in methanol (0.5 mL) and 2,6-lutidine (0.5 mL) was treated with 2(R)-(3-chloro-4-

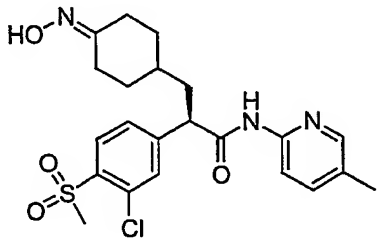
methanesulfonyl-phenyl)-N-(5-methyl-pyrazin-2-yl)-3-(4-oxo-cyclohexyl)-propionamide (prepared as in Example 62, 34 mg, 0.066 mmol). The reaction mixture was stirred at 25°C for 30 min and was then concentrated *in vacuo* to remove methanol. The resulting residue was suspended in ethyl acetate (10 mL), washed with water (1 x 5 mL), dried over magnesium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 12M, Silica, 65/35 hexanes/ethyl acetate to ethyl acetate) afforded 2(R)-(3-chloro-4-methanesulfonyl-phenyl)-3-(4-hydroxyimino-cyclohexyl)-N-(5-methyl-pyrazin-2-yl)-propionamide (75 mg, 100%) was a white foam: $[\alpha]^{23}_{589}$ = –30.0° (c=0.32 chloroform); EI-HRMS m/e calcd for $C_{21}H_{25}ClN_4O_4S$ $(M+H)^+$ 465.1358, found 465.1365.

Example 70
2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-N-(5-chloro-pyridin-2-yl)-3-(4-hydroxyimino-cyclohexyl)-propionamide



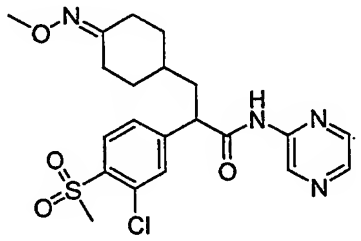
[0282] A solution of hydroxylamine hydrochloride (26.0 mg, 0.36 mmol) in methanol (0.7 mL) and 2,6-lutidine (0.7 mL) was treated with 2(R)-(3-chloro-4-methanesulfonyl-phenyl)-N-(5-chloro-pyridin-2-yl)-3-(4-oxo-cyclohexyl)-propionamide (prepared as in Example 63, 114 mg, 0.24 mmol). The reaction mixture was stirred at 25°C for 30 min and was then concentrated *in vacuo* to remove methanol. The resulting residue was suspended in ethyl acetate (10 mL), washed with water (1 x 5 mL), dried over magnesium sulfate, and concentrated *in vacuo*. Biotage chromatography (FLASH 40S, Silica, 65/35 hexanes/ethyl acetate to 2/8 hexanes/ ethyl acetate) afforded 2(R)-(3-chloro-4-methanesulfonyl-phenyl)-N-(5-chloro-pyridin-2-yl)-3-(4-hydroxyimino-cyclohexyl)-propionamide (105 mg, 90%) was a white powder: $[\alpha]^{23}_{589}$ = –23.03° (c=0.33 chloroform); EI-HRMS m/e calcd for $C_{21}H_{23}Cl_2N_3O_4S$ $(M+H)^+$ 484.0859, found 484.0862.

Example 71
2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-3-(4-hydroxyimino-cyclohexyl)-N-(5-methyl-pyridin-2-yl)-propionamide



[0283] A solution of hydroxylamine hydrochloride (26.0 mg, 0.36 mmol) in methanol (0.7 mL) and 2,6-lutidine (0.7 mL) was treated with 2(R)-(3-chloro-4-methanesulfonyl-phenyl)-N-(5-methyl-pyridin-2-yl)-3-(4-oxo-cyclohexyl)-propionamide (prepared as in Example 64, 105 mg, 0.23 mmol). The reaction mixture was stirred at 25°C for 30 min and was then concentrated *in vacuo* to remove methanol. The resulting residue was suspended in ethyl acetate (10 mL), washed with water (1 x 5 mL), dried over magnesium sulfate, and concentrated *in vacuo*. Biotage chromatography (FLASH 40S, Silica, 65/35 hexanes/ethyl acetate to 2/8 hexanes/ ethyl acetate) afforded 2(R)-(3-chloro-4-methanesulfonyl-phenyl)-3-(4-hydroxyimino-cyclohexyl)-N-(5-methyl-pyridin-2-yl)-propionamide (67 mg, 63%) was a white foam; $[\alpha]^{23}_{589} = -7.84°$ (c=0.37 chloroform); EI-HRMS m/e calcd for $C_{22}H_{26}ClN_3O_4S$ $(M+H)^+$ 464.1406, found 464.1409.

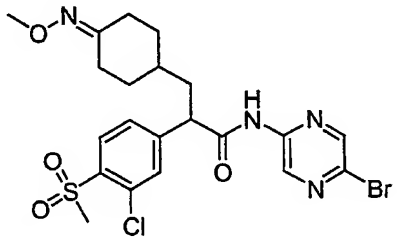
Example 72
2-(3-Chloro-4-methanesulfonyl-phenyl)-3-(4-methoxyimino-cyclohexyl)-N-pyrazin-2-yl-propionamide



[0284] A solution of methoxylamine hydrochloride (23 mg, 0.27 mmol) in methanol (0.5 mL) and pyridine (0.5 mL) was treated with 2-(3-chloro-4-methanesulfonyl-phenyl)-3-(4-oxo-cyclohexyl)-N-pyrazin-2-yl-propionamide (prepared as in

Example 58, 80 mg, 0.18 mmol). The reaction mixture was heated under reflux for 2 h, cooled to 25°C, and concentrated *in vacuo* to remove methanol. The resulting residue was suspended in ethyl acetate (10 mL), washed with water (1 x 5 mL), dried over magnesium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 12M, Silica, 35/65 hexanes/ethyl acetate) afforded 2-(3-chloro-4-methanesulfonyl-phenyl)-3-(4-methoxyimino-cyclohexyl)-N-pyrazin-2-yl-propionamide (68 mg, 81%) was a white foam: EI-HRMS m/e calcd for $C_{21}H_{25}ClN_4O_4S$ $(M+H)^+$ 465.1358, found 465.1364.

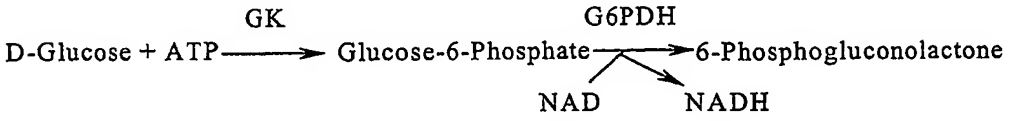
Example 73
N-(5-Bromo-pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(4-methoxyimino-cyclohexyl)-propionamide



[0285] A solution of methoxylamine hydrochloride (16 mg, 0.18 mmol) in methanol (0.5 mL) and pyridine (0.5 mL) was treated with N-(5-bromo-pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(4-oxo-cyclohexyl)-propionamide (prepared as in Example 59, 62 mg, 0.12 mmol). The reaction mixture was heated under reflux for 2 h, cooled to 25°C, and concentrated *in vacuo* to remove methanol. The resulting residue was suspended in ethyl acetate (10 mL), washed with water (1 x 5 mL), dried over magnesium sulfate, filtered, and concentrated *in vacuo*. Biotage chromatography (FLASH 12M, Silica, 7/3 hexanes/ethyl acetate eluted to 4/6 hexanes/ethyl acetate) afforded the N-(5-bromo-pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(4-methoxyimino-cyclohexyl)-propionamide (51 mg, 80%) as a white foam: EI-HRMS m/e calcd for $C_{21}H_{24}BrClN_4O_4S$ $(M+H)^+$ 543.0463, found 543.0464.

Biological Activity Example A: *In Vitro* Glucokinase Activity

[0286] *Glucokinase Assay*: Glucokinase (GK) was assayed by coupling the production of glucose-6-phosphate to the generation of NADH with glucose-6-phosphate dehydrogenase (G6PDH, 0.75-1 kunits/mg; Boehringer Mannheim, Indianapolis, IN) from *Leuconostoc mesenteroides* as the coupling enzyme (Scheme 2). Recombinant



Scheme 2

[0287] Human liver GK1 was expressed in *E. coli* as a glutathione S-transferase fusion protein (GST-GK) [Liang et al, 1995] and was purified by chromatography over a glutathione-Sepharose 4B affinity column using the procedure provided by the manufacturer (Amersham Pharmacia Biotech, Piscataway, NJ). Previous studies have demonstrated that the enzymatic properties of native GK and GST-GK are essentially identical (Liang et al, 1995; Neet et al., 1990).

[0288] The assay was conducted at 25°C in a flat bottom 96-well tissue culture plate from Costar (Cambridge, MA) with a final incubation volume of 120 µl. The incubation mixture contained: 25 mM Hepes buffer (pH, 7.1), 25 mM KCl, 5 mM D-glucose, 1mM ATP, 1.8 mM NAD, 2 mM $MgCl_2$, 1 µM sorbitol-6-phosphate, 1 mM dithiothreitol, test drug or 10% DMSO, 1.8 unit/ml G6PDH, and GK (see below). All organic reagents were >98 % pure and were from Boehringer Mannheim with the exceptions of D-glucose and Hepes that were from Sigma Chemical Co, St Louis, MO. Test compounds were dissolved in DMSO and were added to the incubation mixture minus GST-GK in a volume of 12 µl to yield a final DMSO concentration of 10%. This mix was pre-incubated in the temperature controlled chamber of a SPECTRAmax 250 microplate spectrophotometer (Molecular Devices Corporation, Sunnyvale, CA) for 10 minutes to allow temperature equilibrium and then the reaction was started by the addition of 20 µl GST-GK.

[0289] After addition of enzyme, the increase in optical density (OD) at 340 nm was monitored over a 10 minute incubation period as a measure of GK activity. Sufficient GST-GK was added to produce an increase in OD_{340} of 0.08 to 0.1 units over the 10 minute incubation period in wells containing 10% DMSO, but no test compound. Preliminary experiments established that the GK reaction was linear over this period of time even in the presence of activators that produced a 5-fold increase in GK activity. The GK activity in control wells was compared with the activity in wells containing test GK activators, and the concentration of activator that produced a 50% increase in the activity of GK, i.e., the $SC_{1.5}$, was calculated. All of the compounds of formula I described in the Synthesis Examples had an $SC_{1.5}$ less than or equal to 30 μM.

References:

[0290] Liang, Y., Kesavan, P., Wang, L., Niswender, K., Tanizawa, Y., Permut, M. A., Magnuson, M., and Matschinsky, F. M. Variable effects of maturity-onset-diabetes-of- youth (MODY)-associated glucokinase mutations on the substrate interactions and stability of the enzyme. *Biochem. J.* 309: 167-173, 1995.

[0291] Neet, K., Keenan, R. P., and Tippett, P.S. Observation of a kinetic slow transition in monomeric glucokinase. *Biochemistry* 29;770-777, 1990.

Biological Activity Example B: *In Vivo* Activity

Glucokinase Activator in vivo Screen Protocol

[0292] C57BL/6J mice are orally dosed via gavage with Glucokinase (GK) activator at 50 mg/kg body weight following a two hour fasting period. Blood glucose determinations are made five times during the six hour post-dose study period.

[0293] Mice (n=6) are weighed and fasted for a two hour period prior to oral treatment. GK activators are formulated at 6.76 mg/ml in Gelucire vehicle (Ethanol:Gelucire44/14:PEG400q.s. 4:66:30 v/w/v. Mice are dosed orally with 7.5μl formulation per gram of body weight to equal a 50 mg/kg dose. Immediately prior to dosing, a pre dose (time zero) blood glucose reading is acquired by snipping off a small portion of the animals tail (~1mm) and collecting 15μl blood

into a heparinized capillary tube for analysis. Following GK activator administration, additional blood glucose readings are taken at 1, 2, 4 and 6 hours post dose from the same tail wound. Results are interpreted by comparing the mean blood glucose values of six vehicle treated mice with six GK activator treated mice over the six hour study duration. Compounds are considered active when they exhibit a statistically significant ($p \leq 0.05$) decrease in blood glucose compared to vehicle for two consecutive assay time points.

Galenical Example A

[0294] Tablets containing the following ingredients can be produced in a conventional manner:

Ingredients	mg per tablet
Compound of formula I	10.0 - 100.0
Lactose	125.0
Corn starch	75.0
Talc	4.0
Magnesium stearate	1.0

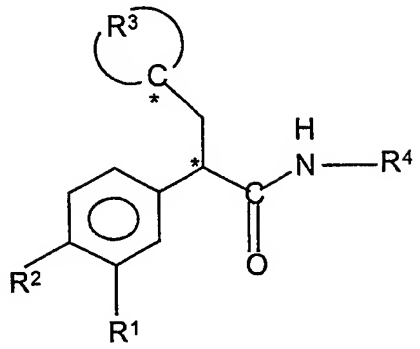
Galenical Example B

[0295] Capsules containing the following ingredients can be produced in a conventional manner:

Ingredients	mg per capsule
Compound of formula I	25.0
Lactose	150.0
Corn starch	20.0
Talc	5.0

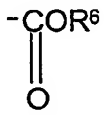
Claims

1. A compound of the formula:



I

wherein R^1 and R^2 are independently hydrogen, halo, amino, hydroxyamino, cyano, nitro, lower alkyl, $-OR^5$,



perfluoro-lower alkyl, lower alkyl thio, perfluoro-lower alkyl thio, lower alkyl sulfonyl, perfluoro-lower alkyl sulfonyl, lower alkyl sulfinyl, or sulfonamido;
R^3 is an unbranched alkyl chain of 4-5 carbon atoms or an unbranched heteroalkyl chain of 3-4 carbon atoms plus one oxygen or sulfur atom, wherein the chain, in combination with the carbon atom it is bonded to, forms a five- or six-membered ring, and

when the chain contains no heteroatoms,

one carbon member of the chain is substituted with one moiety selected from the group consisting of hydroxy, oxo, hydroxyimino, methoxyimino, halo, methoxy, and acetoxy, or

one carbon member of the chain is disubstituted with one hydroxy and one lower alkyl or is disubstituted with halogen

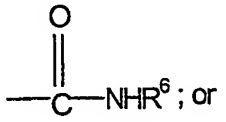
when the chain contains an O heteroatom,

the chain is unsubstituted, and

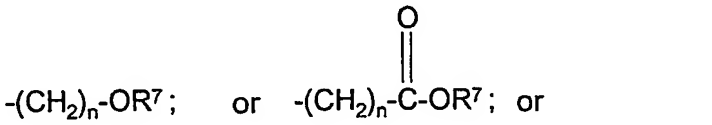
when the chain contains an S heteroatom,

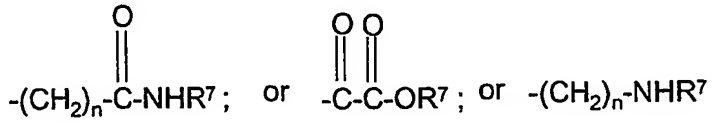
the chain is unsubstituted or the S heteroatom member of the chain is substituted by an oxo group;

R^4 is



an unsubstituted or mono-substituted five- or six-membered heteroaromatic ring connected by a ring carbon atom to the amine group shown, which five- or six-membered heteroaromatic ring contains from 1 to 3 heteroatoms selected from sulfur, oxygen or nitrogen, with one heteroatom being nitrogen which is adjacent to the connecting ring carbon atom; said mono-substituted heteroaromatic ring being mono-substituted at a position on a ring carbon atom other than adjacent to said connecting carbon atom with a substituent selected from the group consisting of lower alkyl, halo, nitro, cyano, perfluoro-lower alkyl, amidooxime, or





n is 0, 1, 2, 3 or 4;

R^5 is hydrogen, lower alkyl, or perfluoro-lower alkyl; R^6 is lower alkyl; and R^7 is hydrogen or lower alkyl;

* denotes a carbon atom that is asymmetric in all or most of the compounds of formula I;

or a pharmaceutically acceptable salt thereof.

2. A compound according to claim 1, wherein R^1 and R^2 are independently hydrogen, halo, perfluoro-lower alkyl, or lower alkyl sulfonyl.

3. A compound according to any of claims 1 to 2, wherein R^1 is hydrogen, halo, or perfluoro-lower alkyl.

4. A compound according to any of claims 1 to 3, wherein R^2 is halo or lower alkyl sulfonyl.

5. A compound according to any of claims 1 to 4, wherein the combination of R^3 and the carbon atom R^3 is attached to are tetrahydrofuranyl, tetrahydropyranyl, tetrahydro-thiopyranyl or cycloalkyl, optionally substituted as defined in claim 1.

6. A compound according to any of claims 1 to 5, wherein the combination of R^3 and the carbon atom R^3 is attached to are tetrahydrofuranyl, tetrahydropyranyl, tetrahydro-thiopyranyl, cyclopentyl, 2-hydroxy-cyclopentyl, 3-hydroxy-cyclopentyl, 4-hydroxy-cyclopentyl, 2-oxo-cyclopentyl, 3-oxo-cyclopentyl, 4-oxo-cyclopentyl, 2-hydroxyimino-cyclopentyl, 3-hydroxyimino-cyclopentyl, 4-hydroxyimino-cyclopentyl, 2-methoxyimino-cyclopentyl, 3-methoxyimino-cyclopentyl, 4-methoxyimino-cyclopentyl, 2-fluorocyclopentyl, 3-methoxy-cyclopentyl, 3-acetoxy-cyclopentyl, 2,2-difluoro-cyclopentyl, 3,3-difluoro-cyclopentyl, or 3-hydroxy-3-methyl-cyclopentyl.

7. A compound according to any of claims 1 to 6, wherein R^4 is $-C(O)NHR^6$ or an unsubstituted or mono-substituted five- or six-membered heteroaromatic ring connected by a ring carbon atom to the amine group shown, which five- or six-membered heteroaromatic ring contains 1 or 2 heteroatoms selected from sulfur and nitrogen, with one heteroatom being nitrogen which is adjacent to the connecting ring carbon atom; said mono-substituted heteroaromatic ring being mono-substituted at a position on a ring carbon atom other than adjacent to said connecting carbon atom with a substituent selected from the group consisting of lower alkyl, halo, cyano, amidooxime, $-(CH_2)_n-OR^7$ or $-(CH_2)_n-C(O)OR^7$.

8. A compound according to any of claims 1 to 7, wherein R^4 is thiazolyl, pyrazinyl, or pyridinyl, optionally mono-substituted at a position on a ring carbon atom other than adjacent to the connecting carbon atom with a substituent selected from the group consisting of methyl, chloro, bromo, cyano, amidooxime, $-(CH_2)_n-OR^7$ and $-(CH_2)_n-C(O)OR^7$.

9. A compound according to any of claims 1 to 8, wherein R^5 is hydrogen, methyl or trifluoromethyl.

10. A compound according to any of claims 1 to 9, wherein R^6 is methyl.

11. A compound according to any of claims 1 to 10, wherein R^7 is hydrogen or methyl.

12. A compound according to any of claims 1 to 11, wherein n is 0 or 1.

13. A compound according to any of claims 1 to 12, selected from the group consisting of:

1-[2-(3,4-Dichloro-phenyl)-3-(tetrahydro-furan-2-yl)-propionyl]-3-methyl-urea,

1-[2-(3,4-Dichloro-phenyl)-3-(2-hydroxy-cyclopentyl)-propionyl]-3-methyl-urea,

1-[2-(3,4-Dichloro-phenyl)-3-(2-oxo-cyclopentyl)-propionyl]-3-methyl-urea,

1-[2-(3,4-Dichloro-phenyl)-3-(3-hydroxy-cyclopentyl)-propionyl]-3-methyl-urea,

1-[2-(3,4-Dichloro-phenyl)-3-(3-oxo-cyclopentyl)-propionyl]-3-methyl-urea,

1-[2-(3,4-Dichloro-phenyl)-3-(tetrahydro-pyran-2-yl)-propionyl]-3-methyl urea,

1-[2-(4-Methanesulfonyl-phenyl)-3-(tetrahydro-pyran-2-yl)-propionyl]-3-methyl-urea,

2-(3,4-Dichloro-phenyl)-3-(tetrahydro-furan-2-yl)-N-thiazol-2-yl-propionamide,

2-(4-Methanesulfonyl-phenyl)-3-(tetrahydro-furan-2(R)-yl)-N-thiazol-2-yl-propionamide,

2-(3,4-Dichloro-phenyl)-3-(2-hydroxy-cyclopentyl)-N-thiazol-2-yl-propionamide,

3-(2-Hydroxy-cyclopentyl)-2-(4-methanesulfonyl-phenyl)-N-thiazol-2-yl-propionamide,

2-(3,4-Dichloro-phenyl)-3-(2-oxo-cyclopentyl)-N-thiazol-2-yl-propionamide,

2-(4-Methanesulfonyl-phenyl)-3-(2-oxo-cyclopentyl)-N-thiazol-2-yl-propionamide,

2-(3,4-Dichloro-phenyl)-3-(3-hydroxy-cyclopentyl)-N-thiazol-2-yl-propionamide,

3-(3-Hydroxy-cyclopentyl)-2-(4-methanesulfonyl-phenyl)-N-thiazol-2-yl-propionamide,

2-(3,4-Dichloro-phenyl)-3-(3-methoxy-cyclopentyl)-N-thiazol-2-yl-propionamide,

3-[2-(3,4-dichloro-phenyl)-2-(thiazol-2-ylcarbamoyl)-ethyl]-cyclopentyl ester,

2-(3,4-Dichloro-phenyl)-3-(3-oxo-cyclopentyl)-N-thiazol-2-yl-propionamide,

2-(4-Methanesulfonyl-phenyl)-3-(3-oxo-cyclopentyl)-N-thiazol-2-yl-propionamide,

2-(3,4-Dichloro-phenyl)-3-(3-fluoro-cyclopentyl)-N-thiazol-2-yl-propionamide,

2-(3,4-Dichloro-phenyl)-3-(3-hydroxyimino-cyclopentyl)-N-thiazol-2-yl-propionamide,

2-(3,4-Dichloro-phenyl)-3-(3-methoxyimino-cyclopentyl)-N-thiazol-2-yl-propionamide,

2-(3,4-Dichloro-phenyl)-3-(2,2-difluoro-cyclopentyl)-N-thiazol-2-yl-propionamide,

2-(3,4-Dichloro-phenyl)-3-(3,3-difluoro-cyclopentyl)-N-thiazol-2-yl-propionamide,

2-(3,4-Dichloro-phenyl)-3-(tetrahydro-pyran-2-yl)-N-thiazol-2-yl-propionamide,

2(R)-(3,4-Dichloro-phenyl)-3-(tetrahydro-pyran-2-yl)-N-thiazol-2-yl-propionamide,

2-(4-Methanesulfonyl-phenyl)-3-(tetrahydro-pyran-2-yl)-N-thiazol-2-yl-propionamide,

2-(4-Methylsulfonyl-3-trifluoromethyl-phenyl)-3-(tetrahydro-pyran-2-yl)-N-thiazole-2-yl-propionamide,

6-[2-(4-Methylsulfonyl-3-trifluoromethyl-phenyl)-3-(tetrahydro-pyran-2-yl)-propionylamino]-nicotinic acid methyl ester,

6-[2-(4-Methylsulfonyl-3-trifluoromethyl-phenyl)-3-(tetrahydro-pyran-2-yl)-propionylamino]-nicotinic acid,

N-(5-Hydroxymethyl-pyridin-2-yl)-2-(4-methylsulfonyl-3-trifluoromethyl-phenyl)-3-(tetrahydro-pyran-2-yl)-propionamide,

2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-N-(5-chloro-pyridin-2-yl)-3-(4-oxo-cyclohexyl)-propionamide,

2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-N-(5-methyl-pyridin-2-yl)-3-(4-oxo-cyclohexyl)-propionamide,

2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-N-(5-chloro-pyridin-2-yl)-3-(4-hydroxyimino-cyclohexyl)-propionamide,

2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-3-(4-hydroxyimino-cyclohexyl)-N-(5-methyl-pyridin-2-yl)-propionamide,

2-(3-Chloro-4-methanesulfonyl-phenyl)-N-pyrazin-2-yl-3-(tetrahydro-furan-2(R)-yl)-propionamide,

2-(4-Methanesulfonyl-3-trifluoromethyl-phenyl)-N-pyrazin-2-yl-3-(tetrahydro-furan-2(R)-yl)-propionamide,

2-(3-Chloro-4-methanesulfonyl-phenyl)-N-pyrazin-2-yl-3-(tetrahydro-furan-3-yl)-propionamide,

2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-3-(2-oxo-cyclopentyl)-N-pyrazin-2-yl-propionamide,

2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-3-(2-hydroxy-cyclopentyl)-N-pyrazin-2-yl-propionamide,

2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-3-(3-hydroxy-cyclopentyl)-N-pyrazin-2-yl-propionamide,

2-(3-Chloro-4-methanesulfonyl-phenyl)-3-(3-oxo-cyclopentyl)-N-pyrazin-2-yl-propionamide,

2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-3-(3-oxo-cyclopentyl)-N-pyrazin-2-yl-propionamide,

2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-3-((S)-3-oxo-cyclopentyl)-N-pyrazin-2-yl-propionamide,

2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-3-((R)-3-oxo-cyclopentyl)-N-pyrazin-2-yl-propionamide,

N-(5-Bromo-pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(3-oxo-cyclopentyl)-propionamide,

2-(3-Chloro-4-methanesulfonyl-phenyl)-3-(2-hydroxyimino-cyclopentyl)-N-pyrazin-2-yl-propionamide,

2-(3-Chloro-4-methanesulfonyl-phenyl)-3-(2-methoxyimino-cyclopentyl)-N-pyrazin-2-yl-propionamide,

2-(3-Chloro-4-methanesulfonyl-phenyl)-3-(3-hydroxyimino-cyclopentyl)-N-pyrazin-2-yl-propionamide,

N-(5-Bromo-pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(3-hydroxyimino-cyclopentyl)-propionamide,

2-(3-Chloro-4-methanesulfonyl-phenyl)-3-(3-methoxyimino-cyclopentyl)-N-pyrazin-2-yl-propionamide,

N-(5-Bromo-pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(3-methoxyimino-cyclopentyl)-propionamide,

2-(3-Chloro-4-methanesulfonyl-phenyl)-3-(3-hydroxy-3-methyl-cyclopentyl)-N-pyrazin-2-yl-propionamide,

2-(4-Methanesulfonyl-3-trifluoromethyl-phenyl)-N-pyrazin-2-yl-3-(tetrahydro-pyran-2-yl)-propionamide,

2-(3-Chloro-4-methanesulfonyl-phenyl)-N-pyrazin-2-yl-3-(tetrahydro-pyran-4-yl)-propionamide,

N-(5-Bromo-pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(tetrahydro-pyran-4-yl)-propionamide,

2-(3-Chloro-4-methanesulfonyl-phenyl)-N-(5-cyano-pyrazin-2-yl)-3-(tetrahydro-pyran-4-yl)-propionamide,

2-(3-Chloro-4-methanesulfonyl-phenyl)-N-[5-(N-hydroxycarbamimidoyl)-pyrazin-2-yl]-3-(tetrahydro-pyran-4-yl)-propionamide,

2-(3-Chloro-4-methanesulfonyl-phenyl)-N-pyrazin-2-yl-3-(tetrahydro-thiopyran-3(R)-yl)-propionamide,

2-(3-Chloro-4-methanesulfonyl-phenyl)-3-(1-oxo-hexahydro-1λ^4-thiopyran-3(R)-yl)-N-pyrazin-2-yl-propionamide,

2-(3-Chloro-4-methanesulfonyl-phenyl)-3-(4-oxo-cyclohexyl)-N-pyrazin-2-yl-propionamide,

N-(5-Bromo-pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(4-oxo-cyclohexyl)-propionamide,

N-(5-Bromo-pyrazin-2-yl)-2(R)-(3-chloro-4-methanesulfonyl-phenyl)-3-(4-oxo-cyclohexyl)-propionamide,

2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-N-(5-chloro-pyrazin-2-yl)-3-(4-oxo-cyclohexyl)-propionamide,

2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-N-(5-methyl-pyrazin-2-yl)-3-(4-oxo-cyclohexyl)-propionamide,

2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-N-(5-chloro-pyrazin-2-yl)-3-(4-hydroxy-cyclohexyl)-propionamide,

2-(3-Chloro-4-methanesulfonyl-phenyl)-3-(4-hydroxyimino-cyclohexyl)-N-pyrazin-2-yl-propionamide,

N-(5-Bromo-pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(4-hydroxyimino-cyclohexyl)-propionamide,

N-(5-Bromo-pyrazin-2-yl)-2(R)-(3-chloro-4-methanesulfonyl-phenyl)-3-(4-hydroxyimino-cyclohexyl)-propionamide,

2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-N-(5-chloro-pyrazin-2-yl)-3-(4-hydroxyimino-cyclohexyl)-propionamide,

2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-3-(4-hydroxyimino-cyclohexyl)-N-(5-methyl-pyrazin-2-yl)-propionamide,

2-(3-Chloro-4-methanesulfonyl-phenyl)-3-(4-methoxyimino-cyclohexyl)-N-pyrazin-2-yl-propionamide,

N-(5-Bromo-pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(4-methoxyimino-cyclohexyl)-propionamide,

and pharmaceutically acceptable salts thereof.

14. A compound according to any of claims 1 to 12, selected from the group consisting of:

N-(5-Bromo-pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(tetrahydro-pyran-4-yl)-propionamide

2-(3-Chloro-4-methanesulfonyl-phenyl)-N-(5-cyano-pyrazin-2-yl)-3-(tetrahydro-pyran-4-yl)-propionamide

2-(3-Chloro-4-methanesulfonyl-phenyl)-N-[5-(N-hydroxycarbamimidoyl)-pyrazin-2-yl]-3-(tetrahydro-pyran-4-yl)-propionamide

2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-3-(3-hydroxy-cyclopentyl)-N-pyrazin-2-yl-propionamide

2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-3-(3-oxo-cyclopentyl)-N-pyrazin-2-yl-propionamide

2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-3-((R)-3-oxo-cyclopentyl)-N-pyrazin-2-yl-propionamide

N-(5-Bromo-pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(3-oxo-cyclopentyl)-propionamide

N-(5-Bromo-pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(3-hydroxyimino-cyclopentyl)-propionamide

2-(3-Chloro-4-methanesulfonyl-phenyl)-3-(3-methoxyimino-cyclopentyl)-N-pyrazin-2-yl-propionamide

N-(5-Bromo-pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(3-methoxyimino-cyclopentyl)-propionamide

N-(5-Bromo-pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(4-oxo-cyclohexyl)-propionamide

N-(5-Bromo-pyrazin-2-yl)-2(R)-(3-chloro-4-methanesulfonyl-phenyl)-3-(4-oxo-cyclohexyl)-propionamide

2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-N-(5-chloro-pyrazin-2-yl)-3-(4-oxo-cyclohexyl)-propionamide

2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-N-(5-methyl-pyrazin-2-yl)-3-(4-oxo-cyclohexyl)-propionamide

N-(5-Bromo-pyrazin-2-yl)-2-(3-chloro-4-methanesulfonyl-phenyl)-3-(4-hydroxyimino-cyclohexyl)-propionamide

N-(5-Bromo-pyrazin-2-yl)-2(R)-(3-chloro-4-methanesulfonyl-phenyl)-3-(4-hydroxyimino-cyclohexyl)-propionamide

2(R)-(3-Chloro-4-methanesulfonyl-phenyl)-N-(5-chloro-pyrazin-2-yl)-3-(4-hydroxyimino-cyclohexyl)-propionamide
and pharmaceutically acceptable salts thereof.

15. A compound according to any of claims 1 to 12 which is 2(R)-(3-chloro-4-methanesulfonyl-phenyl)-3-((R)-3-oxo-cyclopentyl)-N-pyrazin-2-yl-propionamide.

16. A pharmaceutical composition comprising a compound of any of claims 1 to 15 and a pharmaceutically acceptable carrier and/or adjuvant.

17. A process for the preparation of a pharmaceutical composition of claim 16 comprising combining a compound of formula I according to any one of claims 1 to 15 with a pharmaceutically acceptable carrier and/or adjuvant.

18. The compounds according to any of claims 1 to 15 for use as a therapeutic active substance.

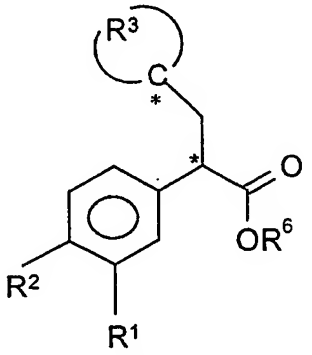
19. The use of the compounds according to any of claims 1 to 15 for the treatment or prophylaxis of type II diabetes.

20. The use of a compound according to any of claims 1 to 15 for the preparation of a medicament for the treatment or prophylaxis of type II diabetes.

21. A method for the prophylactic or therapeutic treatment of type II diabetes, which method comprises administering a compound of any of claims 1 to 15 to a human being or an animal.

22. A process for the preparation of a compound according to any of claims 1 to 15, said process comprising:

a) reacting a compound of formula VIII

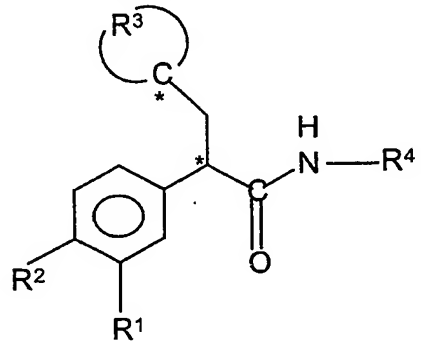


wherein R^1, R^2, R^3 and R^6 are as defined in claim 1;

with a compound of formula X

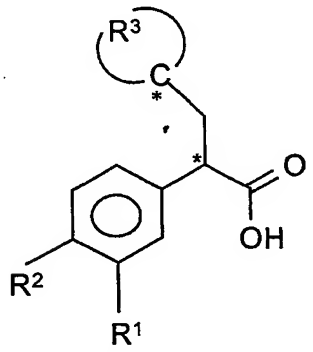
R^4-NH_2

to produce a compound of formula I



wherein R^1, R^2, R^3, R^4 and * are as defined in claim 1;

b) reacting a compound of formula IX

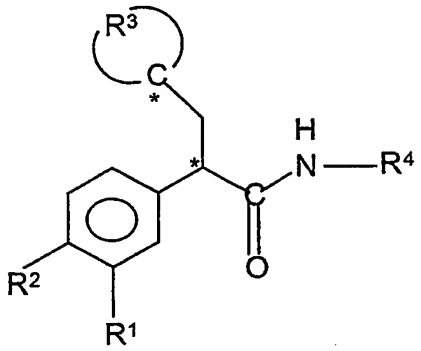


wherein R^1, R^2 and R^3 are as defined in claim 1;

with a compound of formula X

R^4-NH_2

to produce a compound of formula I



wherein R^1, R^2, R^3, R^4 and * are as defined in claim 1.

23. A compound prepared by the processes according to claim 22.

24. The invention as hereinbefore defined.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 03/03844

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D307/16 C07D309/04 C07D417/12 C07D405/12 C07D241/20
C07D409/12 C07D277/46 C07D213/75 C07C275/52 A61K31/335
A61K31/38 A61K31/425 A61K31/4965 A61K31/44 A61P3/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07D C07C A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, BEILSTEIN Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,Y	WO 00 58293 A (F. HOFFMANN-LA ROCHE AG) 5 October 2000 (2000-10-05) the whole document	1-23
Y	WO 02 08209 A (F. HOFFMANN-LA ROCHE AG) 31 January 2002 (2002-01-31) the whole document, particularly examples 4 and 15	1-23

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 July 2003	13/08/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Allard, M

Form PCT/ISA/210 (second sheet) (July 1992)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 24

The scope of claim 24 is so unclear (Article 6 PCT) that a meaningful international search is impossible with regard to this claim.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 03/03844

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 19 and 21 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: 24
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP 03/03844

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0058293 A	05-10-2000	AU 3963000 A	16-10-2000
		BR 0009486 A	02-01-2002
		CA 2368347 A1	05-10-2000
		CN 1349519 T	15-05-2002
		CZ 20013490 A3	17-04-2002
		WO 0058293 A2	05-10-2000
		EP 1169312 A2	09-01-2002
		HU 0200396 A2	29-07-2002
		JP 2002540196 T	26-11-2002
		NO 20014671 A	26-09-2001
		NZ 514038 A	28-09-2001
		PL 350669 A1	27-01-2003
		TR 200102805 T2	22-04-2002
		US 2001039344 A1	08-11-2001
		US 6528543 B1	04-03-2003
WO 0208209 A	31-01-2002	AU 8760001 A	05-02-2002
		BR 0112658 A	24-06-2003
		CA 2416229 A1	31-01-2002
		WO 0208209 A1	31-01-2002
		EP 1305301 A1	02-05-2003
		US 2002198200 A1	26-12-2002
		US 2002042512 A1	11-04-2002

Specification

Crystal of glucokinase protein and drug design method using crystal thereof.

The Field of Technology

This invention relates to crystal of novel glucokinase protein (hereinafter it is called "GK protein") and the drug design method or the like employing three-dimensional structure coordinates obtained by using the crystal thereof.

Background Technique

Glucokinase (ATP: D-hexose 6-phosphotransferaze, EC2.7.1.1) is one of four kinds of hexokinase isozymes of mammals (hexokinase IV). These isozymes catalyse the same reaction, however, differences exist in the Km value with respect to glucose. In other words, the Km value of hexokinase I, II and III being 10^{-6}-10^{-4} M, but on the other hand the Km value of hexokinase IV, called glucokinase with respect to glucose is much greater at about 10^{-2} M. Hexokinase is an enzyme participating in the initial stage of glycolytic pathway, and catalyses the reaction from the glucose to glucose-6-phosphate.

As for glucokinase, the expression is mainly localised in liver and pancreatic beta cell, and it plays an important role in glucose metabolism of the whole body by controlling the rate-determining step of glucose metabolism in these cells. As for the glucokinase of liver and pancreatic beta cell, the sequence of 15 amino acids at N terminal is respectively different due to splicing difference. However, the enzymatic characteristics are the same.

The hypothesis that glucokinase acts as glucose sensor of pancreatic beta cell and liver is proposed since approximately 10 years ago (Garfinkel D, et al: Am J Physiol 247 [3Pt2]: R527-536, 1984). In practice, it is becoming clear from results of recent glucokinase gene manipulation mouse, that the glucokinase plays an important role in glucose homeostasis of the whole body.

The mouse in which glucokinase gene is destroyed dies of diabetes mellitus soon after birth (Grupe A, et al: Cell 83: 69-78. 1995). On the other hand, as for the mouse which overexpressed glucokinase, the blood glucose level becomes low (Ferre T, et al: Proc Natl Acad Sci USA 93: 7225-7230. 1996). When glucokinase activity is increased by the rise in glucose concentration, although the reactions of

pancreatic beta cell and hepatocyte are different, in each case, it acts in the direction of lowering blood glucose. The pancreatic beta cell starts to secrete more insulin, the liver takes up sugar and stores as glycogen and at the same time lowers the sugar release.

In this way, the fluctuation of glucokinase enzyme activity plays an important role in glucose homeostasis of mammal through liver and pancreatic beta cell. Glucokinase gene mutation is discovered in the case that develops diabetes mellitus in youth known as MODY2 (maturity-onset diabetes of the young), and the lowering of glucokinase activity is said to be the cause of blood glucose rise (Vionnet N, et al.: Nature 356: 721-722, 1992). On the other hand, the lineage having mutation to increase glucokinase activity is also found, and such persons show hypoglycemic symptom (Glaser B, et al.: N Engl J Med 338: 226-230, 1998).

From the above, glucokinase also acts glucose sensor in human and plays an important role in glucose homeostasis. On the other hand, because the glucokinase of many type II diabetics is not mutated, the blood glucose control using glucokinase sensor system is considered possible. Because the glucokinase activator substance can be expected to have insulin secretion promotion action of pancreatic beta cell and sugar up take acceleration and sugar release suppression actions in liver, it is considered as useful therapeutic drug of type II diabetic patients.

Recently, a localised expression of pancreatic beta cell type glucokinase was found in rat brain, in particular in ventromedial hypothalamic nucleus (Ventromedial hypothalamus, VMH) which is the feeding centre. About 20 % of neurons of VMH was known as glucose responsive neuron, and it has been considered to play an important role in weight control in the past. When glucose is administered intracerebrally to rat, food consumption falls, whereas when the glucose metabolism is suppressed by administration of glucose analogue, glucosamine in brain, overeating occurs. From electrophysiological experiment, glucose responsive neurons are found to be activated in response to physiological glucose concentration changes (5-20 mM), however its activity is suppressed when the glucose metabolism is inhibited with glucosamine and the like. In glucose concentration sensing system of VMH, a mechanism through glucokinase the same as insulin secretion of pancreatic beta cells is assumed. Accordingly, there is a possibility that a substance that causes glucokinase activation of VHM in addition to liver, pancreatic beta cell is expected to correct problem of obesity which is a problem in many type II diabetic patients,

in addition to blood glucose correction effect.

On the other hand, it is described in DIABETES, vol. 48, 1698-1705, September 1999 that the stereostructure of glucokinase was predicted from hexokinase 1. However, in practice, crystallisation was not carried out, nor it was a practical one.

In accordance with the above, to elucidate three-dimensional stereostructure of glucokinase and to enable efficient discovery of a compound that interacts with glucokinase are thought to greatly contribute to the development of for example a therapeutic agent or preventative agent of diabetes, a therapeutic agent or preventative agent of chronic complication of diabetes mellitus such as retinopathy, nephropathy, neurosis, ischemic cardiac disease, arteriosclerosis or the like, a therapeutic agent or preventative agent of obesity.

Presently, CARDD (Computer Aided Rational Drug Design) using computer for the tasks such as analysis of active centre of a protein or a prediction of reaction mechanism has been employed at practical level.

In the drug creation system using CARDD, the structure of active site of protein is predicted based on the three-dimensional structure analysis data of the target protein. And information about candidate compounds which can bind to the structure of active site thereof is obtained from the compound database. Thereafter, on consideration of the three-dimensional structure and physical properties of the active site of the target protein and the candidate compound, candidate compounds which can bind to the target protein are selected. These steps are so-called in silico screening step.

Whether the compound selected by in silico screening step binds to the target protein and change the activity thereof or not, is examined by actual examination (wet experiment). And the compound that changes the activity of the target protein becomes the effective ingredient of a drug. Thereby a compound that interacts with the target protein can be efficiently screened without carrying out the procedure wherein innumerable compounds are acted on the target protein one by one and the interactions are confirmed.

In silico screening can be said as an effective means of pharmaceutical development because the

candidate compounds that bind to the target protein can be greatly narrowed down.

Three-dimensional structure analysis data by X-ray structure analysis of the target protein becomes an important information in drug creation system using CARDD. Crystal of target protein is required as analysis sample in three-dimensional structural analysis by X-ray structure analysis. Accordingly, in order to carry out development of drug creation related to GK based on the drug creation system using CARDD, the crystal of GK is required. However, as stated above, crystallisation of GK was difficult, and it could not provide information necessary for CARDD.

This invention was made on consideration of the problems of aforesaid technology of the prior art, and had objects to obtain crystal of glucokinase and to design compounds that bind to glucokinase based on the information obtained from aforesaid crystal.

Disclosure of the Invention

At least one of aforesaid objects is solved by the following invention.

[1] A glucokinase protein characterised in being used for crystallisation.

[2] A protein in accordance with aforesaid [1] comprising amino acid sequence in accordance with Sequence Number 5.

[3] A crystal of protein comprising amino acid sequence in accordance with Sequence Number 5 or amino acid sequence substantially the same amino acid sequence thereof.

[4] A crystal in accordance with aforesaid [3], wherein the said protein is glucokinase protein.

[5] A crystal in accordance with aforesaid [3] comprising crystals of protein containing amino acid sequence in accordance with Sequence Number 5.

[6] A crystal in accordance with aforesaid [3], wherein the lattice constant satisfies the following equations (1)-(4)

a = b = 79.9 +/- 4 Å (1)

c = 322.2 +/- 15 Å (2)

alpha = beta = 90° (3)

gamma = 120° (4)

[7] A crystal in accordance with aforesaid [6], wherein the space group is $P6_522$.

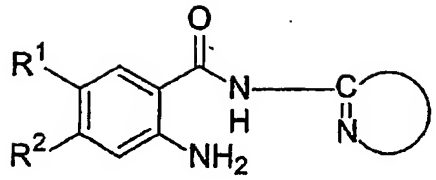
[8] A crystal of protein specified by three-dimensional structure coordinates data in accordance with Table 1.

[9] A crystal wherein in three-dimensional structure coordinates data changed in at least one data of three-dimensional structure coordinates data in accordance with Table 1, the mean square error between atoms of main chain of amino acid represented by three-dimensional structure coordinates data in accordance with Table 1 (C alpha atom) and C alpha atoms represented by the said changed three-dimensional structure coordinates data corresponding to aforesaid C alpha atoms is 0.6 Å or less.

[10] A crystal in accordance with any of [3]-[9], wherein the compound binding site is constructed by at least one of amino acid residues of tyrosine 61 - serine 69, glutamic acid 96 - glutamine 98, isoleucine 159, methionine 210 - tyrosine 215, histidine 218 - glutamic acid 221, methionine 235, arginine 250, leucine 451 - lysine 459 in amino acid sequence shown in sequence Number 5.

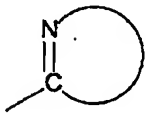
[11] A crystal including a complex of the protein comprising amino acid sequence in accordance with Sequence Number 5 or amino acid sequence substantially the same amino acid sequence thereof and a compound which can bind to the said protein.

[12] A crystal in accordance with aforesaid [11], wherein aforesaid compound is represented by formula (1).



(I)

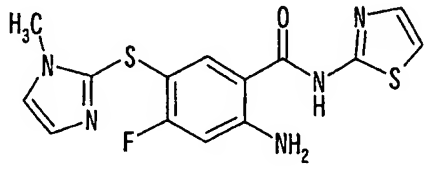
[wherein, R1 shows halogen atom, -S-(O)p-A, -S-(O)q-B or -O-B (wherein, p and q are the same or different and denote an integer of 0-2, A denotes C1-C6 alkyl group of optionally substituted straight chain, B denotes optionally substituted five-membered or six-membered ring aryl group or heteroaryl group, R2 denotes a hydrogen atom or halogen atom, and



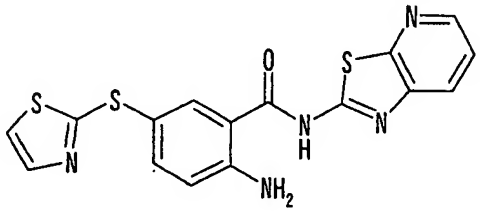
(II)

denotes an optionally substituted monocyclic or bicyclic heteroaryl group having a nitrogen atom adjacent to the carbon atom bonded to amide group].

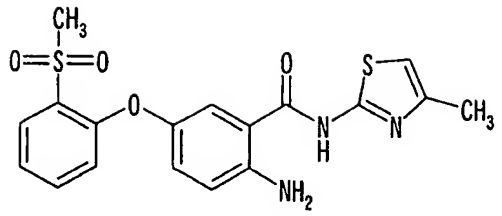
[13]. A crystal in accordance with aforesaid [12], wherein aforesaid compound is any of the compound represented by formula (IIIa)-(IIIc).



(IIIa)



(IIIb)



(IIIc)

[14] A protein in accordance with aforesaid [1] comprising amino acid sequence in accordance with Sequence Number 8.

[15] A crystal of protein comprising amino acid sequence in accordance with Sequence Number 8 or amino acid sequence substantially the same amino acid sequence thereof.

[16] A crystal in accordance with aforesaid [15], wherein the said protein is glucokinase protein.

[17] A crystal in accordance with aforesaid [15] comprising crystals of protein containing amino acid sequence in accordance with Sequence Number 8.

[18] A crystal in accordance with aforesaid [15], wherein the lattice constant satisfies the following equations

a = b = 103.2 +/- 5 Å (5)

c = 281.0 +/- 7 Å (6)

alpha = beta = 90° (7)

gamma = 120° (8)

[19] A crystal in accordance with aforesaid [18], wherein the space group is $P6_522$.

[20] A crystal of protein specified by three-dimensional structure coordinates data in accordance with Table 2.

[21] A crystal wherein in three-dimensional structure coordinates data changed at least one data of three-dimensional structure coordinates data in accordance with Table 2, the mean square error between atoms

of main chain of amino acid represented by three-dimensional structure coordinates data in accordance with Table 2 (C alpha atom) and C alpha atoms represented by the said changed three-dimensional structure coordinates data corresponding to aforesaid C alpha atoms is 0.6 Å or less.

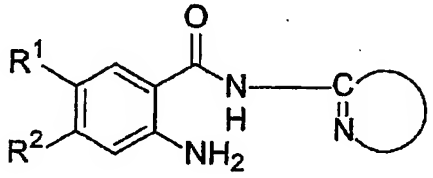
[22] A process for the production of crystal containing a complex of protein and a compound that binds to the protein thereof, including

a protein production step wherein a protein containing the amino acid sequence having deletion of 1-50 amino acid residues from either or both of N terminal and C terminal of the protein containing amino acid sequence in accordance with Sequence Number 2 is produced, and

a protein reaction step wherein a compound that binds to the protein obtained in the said protein production step and the protein obtained in the said protein production step are reacted.

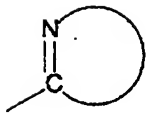
[23] A process to produce crystal of the kind wherein a crystal of a protein is produced, characterised in that a protein including amino acid sequence in accordance with Sequence Number 5 or amino acid sequence substantially the same amino acid sequence thereof and having glucokinase activity and a compound which can bind to the said protein are used.

[24] A process for the production of crystalline protein in accordance with aforesaid [23], wherein the compound which can bind to said protein is a compound represented by formula (1).



(I)

[wherein, R1 shows halogen atom, -S-(O)p-A, -S-(O)q-B or -O-B (wherein, p and q are the same or different and denote an integer of 0-2, A denotes C1-C6 alkyl group of optionally substituted straight chain, B denotes optionally substituted five-membered or six-membered ring aryl group or heteroaryl group, R2 denotes a hydrogen atom or halogen atom, and



(II)

denotes an optionally substituted monocyclic or bicyclic heteroaryl group containing nitrogen atom adjacent to the carbon atom bonded to amide group].

[25]. A process for the production of crystal in accordance with aforesaid [23] or [24] using co-crystallisation or soaking method

[26] A drug design method of the kind wherein based on stereostructural information of a protein, the structure of compound that binds to said protein is designed, characterised in that the stereostructure information of said protein is the information obtained by analysing crystal in accordance with any one of aforesaid [3]-[13] or [15]-[21].

[27] A drug design method in accordance with aforesaid [26] characterised in that
a binding site deduction step wherein the compound binding site of said protein is deduced based on aforesaid stereostructure information, and
a selection step wherein a compound compatible to the compound binding site deduced in aforesaid binding site deduction step is selected from compound library,
are included.

[28] A drug design method in accordance with aforesaid [26] characterised in that
a binding site deduction step wherein the compound binding site of said protein is deduced based on aforesaid stereostructure information, and
a compound structure assembly step wherein the structure of compound compatible to compound binding site deduced in aforesaid binding site deduction step is constructed,
are included.

[29] A drug design method in accordance with aforesaid [26] characterised in that

a binding site deduction step wherein the compound binding site of said protein is deduced based on aforesaid stereostructure information, and

a design step wherein the structure of compound is designed by visual observation so that the compound binding site deduced in aforesaid binding site deduction step and a compound compatible to said compound binding site interact,

are included.

[30] A drug design method in accordance with any of aforesaid [26]-[29], wherein aforesaid compound binding site is constituted by at least one of amino acid residue of tyrosine 61 - serine 69, glutamic acid 96 - glutamine 98, isoleucine 159, methionine 210 - tyrosine 215, histidine 218 - glutamic acid 221, methionine 235, arginine 250, leucine 451 - lysine 459 in amino acid sequence shown in sequence Number 5.

[31] A drug design method in accordance with any of aforesaid [26]-[30] further including a step to measure physiological activity of the candidate compound predicted to be compatible to aforesaid compound binding site.

[32] A drug design method in accordance with any of aforesaid [26]-[30] further including a binding determination step wherein the candidate compound predicted to be compatible to aforesaid compound binding site and a protein including amino acid sequence in accordance with and Sequence Number 5 or amino acid sequence which is substantially the same amino acid sequence thereof are contacted, and whether the candidate compound binds to the said protein or not is assessed.

[33] A process for the production of compound array including the compound group selected by drug design method in accordance with any of aforesaid [26]-[30] is combined as compound array.

Brief Description of the Figures

Figure 1 is a ribbon diagram showing three-dimensional structure of glucokinase.

(Figure 1a is a ribbon diagram showing the structure of glucokinase (Δ1-11)/glucose/compound 1 (compound of formula IIIa). Moreover, the figure on the right is a rotated figure of the figure on the left.)

(Figure 1b is a ribbon diagram showing the simple substance of glucokinase [ΔI-15]. Moreover, the figure on the right is a rotated figure of the figure on the left.)

Figure 2 is a figure showing coupling scheme of compound 1 (compound of formula IIIa) with respect to the binding site of glucokinase (ΔI-11)

Figure 3 is a figure showing the structure of binding site of glucokinase (ΔI-11).

Ideal form for Carrying Out the Invention

In this specification, amino acids, peptides and proteins are represented using abbreviations adopted from the IUPAC-IUB biochemistry designation committee (CBN) shown below. Moreover, the sequence of amino acid residues of peptide and protein are represented so that the N terminal to C terminal comprises from the left end to the right end and moreover the N terminal comprises the first.

Hereinafter, each embodiment of this invention is described in greater detail.

(Glucokinase protein).

Firstly, this invention puts forward glucokinase protein characterised in being used for crystallisation. Glucokinase protein (GK protein) is involved in extremely important sugar metabolism in vivo as described above. Accordingly, by solving the three-dimensional structure of GK protein and by elucidating active site of GK protein, it is possible to search compounds that bind to GK protein (activator or inhibitor). Therefore it is important to clarify the three-dimensional structure of GK protein.

As technique to clarify the three-dimensional structure of protein, X-ray crystal structure analysis is well known. In other words, protein is crystallised, mono-chromatised X-ray is irradiated to the crystal thereof, and three-dimensional structure of said protein is elucidated on the basis of the obtained X-ray diffraction pattern (Blundell, T.L. and Johnson, L.N, PROTEIN CRYSTALLOGRAPHY, pp. 1-565, (1976) Academic Press, New York). First GK protein needs to be crystallised in order to provide for the x-ray crystal structure analysis of GK protein.

Wherein, the "GK protein" of this invention refers to human derived liver type glucokinase having amino acid sequence shown in sequence Number 2 and a protein containing amino acid sequence which is substantially the same as Sequence Number 2. Wherein, as aforesaid protein containing amino acid sequence which is substantially the same, a species having glucokinase activity is preferable. Accordingly, in this specification, the GK protein includes not only the human derived liver type glucokinase, however also human derived pancreas type glucokinase, and non-human derived GK proteins such as mouse, rat, monkey and the like. In this invention human liver type glucokinase is preferably used. In glucokinase derived from human, 15 amino acid residues at N terminal differ in the liver type and the pancreas type. Wherein, "glucokinase activity" refers to an activity to catalyse reaction from glucose to glucose-6-phosphate.

It is generally well known that the crystallisation of protein is difficult, and the GK protein was not able to be crystallised without treatment. These inventors carried out various investigations with trial and error, as a result succeeded in crystallisation of GK protein for the first time by deletion of 11 or 15 amino acids at the N terminal side of GK protein. It was thought that the deleted region protruded from the globular GK protein molecule when the crystallisation was attempted, as a result, caused steric hindrance between adjacent GK protein molecules, and prevented the crystallisation of the GK protein. In other words, in this invention, the crystal of GK protein was obtained by using a GK protein in which 11 amino acid residues at N terminal side is deleted (Sequence Number 5) or a GK protein in which 15 amino acid residues at N terminal side is deleted (Sequence Number 8) in the glucokinase in which amino acids sequence had been known however the crystallisation had been unsuccessful. Wherein the number of amino acids is not restricted as long as it is within a range that the steric hindrance disappears between adjacent crystals. In an embodiment for example, in amino acid sequence represented by Sequence Number 2, amino acids sequence or the like in which amino acid residues of 1-50, preferably 3-30, more preferably 5-25, more preferably still 8-18, most preferably 11-15 at N terminal side are deleted, can be used in this invention. Moreover, the amino acid sequence or the like in which amino acid residues of 1-8, preferably 1-7, more preferably 2-6 at C terminal side are deleted, is used in this invention.

(Crystal of glucokinase protein and a process for the production thereof).

Next, in this invention, crystals including protein containing amino acids sequence in accordance with Sequence Number 5, and Sequence Number 8 or amino acids sequence which is substantially the same as

amino acid sequence thereof are put forward.

As described earlier, as GK protein used in crystallisation, proteins containing amino acids sequence in accordance with Sequence Number 5, and/or Sequence Number 8 or amino acids sequence which is substantially the same as amino acid sequence thereof, or the like are used.

The proteins containing amino acids sequence in accordance with Sequence Number 5, and/or Sequence Number 8 or amino acids sequence which is substantially the same as the amino acid sequence thereof (hereinafter it may be abbreviated as "GK protein" together with proteins containing amino acids sequence in accordance with Sequence Number 2 or amino acids sequence which is substantially the same as the amino acid sequence thereof) can be any as long as crystallisation is possible, and the amino acid sequence thereof is not restricted in particular. Wherein, the proteins containing amino acids sequence which is substantially the same as the amino acid sequence in accordance with Sequence Number 5, and/or Sequence Number 8 does not necessarily have glucokinase activity, and may be an inactive mutant (for example, a mutant inactivated by the presence of mutation at ATP binding site) as long as it has a crystal structure from which the information necessary for drug design can be obtained. Wherein, as proteins containing amino acids sequence which is substantially the same as the amino acid sequence in accordance with Sequence Number 2 or Sequence Number 5, amino acids sequences having about 60 % or more, preferably about 70% or more, more preferably about 80% or more, in particular preferably about 90% or more, and most preferably about 95% or more homology to the amino acids sequence in accordance with Sequence Number 2 or Sequence Number 5, or the like are nominated. Moreover, as proteins containing amino acids sequence which is substantially the same as the amino acids sequence in accordance with Sequence Number 2 or Sequence Number 5, for example, amino acids sequences in which amino acid residues of 1-10, preferably 1-5, more preferably 1-3 more preferably still 1-2 are substituted, deleted, added and/or inserted in the amino acids sequence in accordance with Sequence Number 2 or Sequence Number 5 are exemplified.

Three-dimensional structural analysis of GK protein is carried out for example as follows. Firstly, the protein is purified. And a series of steps such as crystallisation, X-ray diffraction intensity data collection, phase determination of each diffraction spot, electron density calculation, molecular model construction, refinement of structure or the like is carried out. As main equipment for performing

protein structure analysis, incubator for crystallisation, binocular microscope, X-ray diffractometer, three dimensional computer graphics apparatus or the like are used. The actual experimental process to produce protein crystals is divided into step to purify protein in large amount (several mg or more is preferred), a step to widely search conditions for obtaining crystal and a step to obtain high quality crystal suitable for X-ray analysis. Hereinafter, each step is described in concrete terms.

For crystallisation, GK protein is purified to high purity. As process for purification, well known processes can be used, and for example, column chromatography, salt precipitation, centrifugation or the like are used.

Purified GK protein is crystallised and provided as a sample for X-ray crystal structure analysis. Crystallisation is performed based on well known method such as vapor diffusion method, dialysis or the like. When obtaining protein crystals, many elements such as purity / concentration of protein, temperature, pH, concentration of the precipitant used need to be examined. Investigation of crystallisation conditions can be carried out over a wide range using commercial screening reagent, and it is preferably screened using 1-2 μl of protein solution in protein concentration of 1-2 % per condition. In this way when microcrystals or the like are obtained, it is preferred to further refined the conditions.

Moreover, extremely many conditions must be searched in order to obtain crystal of GK protein. Accordingly, a large quantity expression system of the protein is preferably constructed also for the investigation of crystallisation condition. Generally, among proteins, many of the crystallising species are monodispersed in solution state, and polydispersed species do not crystallise in most cases. Therefore, N terminal of GK protein is successively removed, monodispersion properties of protein solution are assessed for the obtained protein using light scattering apparatus, and whether sample is suitable for crystallisation or not may be examined.

Next, using the obtained crystal of GK protein, X-ray diffraction intensity measurement is carried out. Recently, a method wherein the crystal is scooped with a ring of narrow thread or the like, is rapidly cooled to liquid nitrogen temperature, and is measured at low temperature as it is, may also be used. Usually, the intensity measurement of diffracted x-ray is performed by two-dimensional detector such as image plate or the like. Many diffraction lines generated by rotating crystal while irradiating the X-ray

are recorded on image plate, and the recorded diffraction intensities are read by shining a laser.

Next, it is preferred to prepare heavy atom iso-form replacement bodies by heavy atom soaking method or co-crystallisation method. Using this, the phase of the protein crystal can be determined by multiple isomorphous replacement method (MIR method). Instead of introducing heavy atom, the phase is also determined by multiwavelength anomalous scattering method (MAD method) based on the diffraction intensity data using complex X-rays. Molecular replacement method (MR method) in which, when a structure of molecule containing analogous structure has been already solved, the initial structure can be obtained by applying the molecular structure thereof in the crystal, Furrier synthesis diagram is drawn on the basis of this, and the structure of remaining part is elucidated, and the total structure is determined, is known as well.

Once the phase was determined by aforesaid process, electron density is determined from this. The precision of this depends on the number of reflection (resolution) and the precision of the reflection used. The resolution is expressed with the minimum plane spacing of the reflection used. Molecular model is constructed from this electron density diagram. When the molecular model is constructed, the atomic coordinates are obtained, therefore, the calculated value of structure factor is determined from this, and refinement of atomic parameters is carried out by the least-square method to approximate this size to the observed value. In this way, the most reasonable structural information is obtained.

In accordance with this invention, the crystal of GK protein shown in sequence Number 5 has been successfully prepared (cf. later described Example). The obtained crystal of GK protein had lattice constant which satisfied the following equations (1)-(4).

a = b = 79.9 +/- 4 Å (1)

c = 322.2 +/- 15 Å (2)

alpha = beta = 90° (3)

gamma = 120° (4)

Moreover, this crystal was elucidated to have space group $P6_522$. Wherein, aforesaid a = b is preferably 79.9 +/- 3 Å, more preferably 79.9 +/- 2 Å and even more preferably 79.9 +/- 1 Å. Moreover, aforesaid c is preferably 322.2 +/- 10 Å, more preferably 322.2 +/- 8 Å, and even more preferably 322.2 +/- 5 Å.

The three-dimensional structural coordinates of the GK protein crystal obtained in this way are shown in Table 1.

Table 1

Moreover, Table 1 is constructed in accordance with representation method of protein data bank generally used by a person skilled in the art. The GLC in Table 1 denotes glucose molecule, and CP1 denotes the compound represented by formula IIIa, and HOH denotes water molecule.

Moreover, in this invention, the crystal of GK protein shown in sequence Number 8 has been successfully prepared (cf. later described Example). The obtained crystal of GK protein had lattice constant which satisfied the following equations (5)-(8).

a = b = 103.2 +/- 5 Å (5)

c = 281.0 +/- 7 Å (6)

alpha = beta = 90° (7)

gamma = 120° (8)

Moreover, this crystal was elucidated to have space group $P6_522$. Wherein, aforesaid a = b is preferably 103.2 +/- 3 Å, more preferably 103.2 +/- 2 , and even more preferably 103.2 +/- 1 Å. Moreover, aforesaid c is preferably 281.0 +/- 6 Å, more preferably 281.0 +/- 4 Å, and even more preferably 281.0 +/- 2 Å.

The three-dimensional structural coordinates of the GK protein crystal obtained in this way are shown in Table 2.

Table 2

Moreover, Table 2 is constructed in accordance with representation method of protein data bank generally used by a person skilled in the art. The HOH in Table 2 denotes water molecule.

In this invention, crystals of the protein having amino acids sequence which is substantially the same as Sequence Number 5 and/or Sequence Number 8 and having glucokinase activity are within the range of

this invention. As such crystals, for example, crystals wherein in three-dimensional structure coordinates data changed at least one data of three-dimensional structure coordinates data in accordance with Table 1 and/or 2, the mean square error between atoms of main chain of amino acid represented by three-dimensional structure coordinates data in accordance with Table 1 and/or 2 (C alpha atom) and C alpha atoms represented by the said changed three-dimensional structure coordinates data corresponding to aforesaid C alpha atoms is 0.6 Å or less, are nominated. Even if the numerical values of coordinates representing the position of atoms differ, two structural coordinates which can superimpose corresponding atoms contained in the structural coordinates on top of one another show the same three-dimensional structure.

Moreover, the three-dimensional structural coordinates of GK protein in accordance with Table 1 and/or Table 2 are important information for drug design, and stored in a storage medium that can be read by computer in accordance with requirements, this information is processed with computer, and drug design is carried out. Accordingly, in another embodiment of this invention, a computer readable recording medium which recorded a program to function a computer as three dimensional coordinate memory means that memorises three-dimensional coordinates of amino acid residue in accordance with Table 1 and/or 2 is put forward.

Moreover, according to another embodiment of this invention, a computer readable recording medium that recorded a program which functions using computer as three dimensional coordinates memory means that memorised the three-dimensional coordinates of amino acid residue in accordance with Table 1 and/or 2, as binding site deduction means that deduces compound binding site of a protein having amino acid sequence represented by Sequence Number 8 and/or Sequence Number 5 using three dimensional coordinates of amino acid residue in accordance with Table 1 and/or 2 memorised in aforesaid three-dimensional coordinates memory measure, as binding compound memory means which memorised information about the type of compounds that bind to the protein and three-dimensional structure of aforesaid compounds, and as binding compound candidate selection means for selecting candidate compounds which are compatible to the compound binding site of the protein having amino acid sequence represented by Sequence Number 1 at least using the information about the three-dimensional structure of compound binding site of protein containing amino acid sequence represented by Sequence Number 8 and/or inferred Sequence Number 5 deduced by aforesaid binding site deduction means and the

information about three-dimensional structure of compound memorised in aforesaid binding compounds memory means, is put forward. Moreover, according to another embodiment of this invention, a computer equipped with aforesaid each means, is also put forward.

(Crystal of complex of GK protein with compound that binds to this).

Next, according to another embodiment of this invention, a crystal containing a complex of protein including amino acid sequence in accordance with Sequence Number 5 or Sequence Number 8 or amino acid sequence which is substantially the same amino acid sequence thereof with the compound which can bind to the said protein and a process for the production thereof are put forward.

When a compound which binds to GK protein is obtained, firstly, the GK protein and the compound thereof are mixed for example in an aqueous solution, and a complex is formed. As for the crystal of such complex, well known processes for the production of co-crystals such as co-crystallisation, soaking method or the like are used. As for the crystallisation condition and crystallisation process, refer to aforesaid processes.

For example, a compound that binds to GK protein is selected from the compound group represented by aforesaid formula (I).

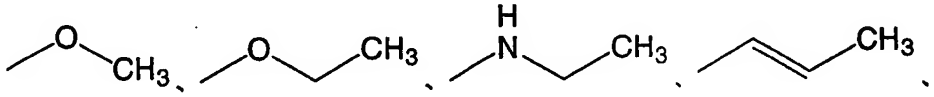
Wherein, as halogen atom of aforesaid formula (I), fluorine atom, chlorine atom, bromine atom, iodine atom or the like are exemplified, and among these, chlorine atom is preferred.

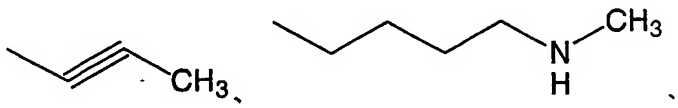
Moreover, as far as substituents in heteroaryl group of A, B of aforesaid formula (1) and formula (II) are concerned, amino group, carbamoyl group, carbamoyl amino group, carbamoyloxy group, carboxyl group, cyano group, sulphamoyl group, trifluoromethyl group, halogen atom, hydroxy group, formyl group, straight chained C1-C6 alkyl group, cyclic C3-C6 hydrocarbon group, aralkyl group, N-aralkyl amino group, N,N-diaralkyl amino group, aralkyloxy group, aralkyl carbonyl group, N-aralkyl carbamoyl group, aryl group, arylthio group, N-arylamino group, aryloxy group, aryl sulphonyl group, aryl sulphonyloxy group, N-arylsulfonylamino group, aryl sulphamoyl group, N-aryl carbamoyl group, aroyl group, aroxy group, C2-C6 alkanoyl group, N-C2-C6 alkanoyl amino group, C1-C6 alkylthio group, N-C1-C6 alkyl sulphamoyl group, N,N-di-C1-C6 alkyl sulphamoyl group, C1-C6 alkyl sulfinyl group, C1-

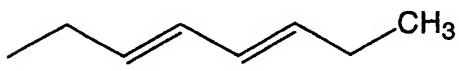
C6 alkylsulfonyl group, N-C1-C6 alkylsulfonyl amino group, C1-C6 alkoxy group, C1-C6 alkoxycarbonyl group or C1-C6 alkylamino group are denoted), or the like is nominated. Wherein, as for the preferably used substituent, amino group, carbamoyl group, carbamoyl amino group, carbamoyloxy group, carboxyl group, cyano group, sulphamoyl group, trifluoromethyl group, halogen atom, hydroxy group, formyl group, straight chained C1-C6 alkyl group or the like are exemplified.

Wherein, "hydrocarbon group" denotes 1-6 C straight chained alkyl group, or, among carbon atom constituting said alkyl group, a group in which 1 or 2, preferably 1 carbon atom may be substituted with nitrogen atom, sulfur atom or oxygen atom and/or carbon atom themselves in the said 1-6 C straight chain alkyl group may be bonded with double bond or triple bond. Number of said double bond or triple bond is preferably 1 or 2 and 1 is more preferred.

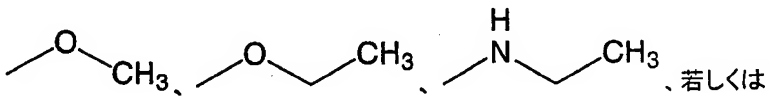
As said hydrocarbon group, in an embodiment, it is preferred to be methyl group, ethyl group, propyl group or isopropyl group, butyl group or a group represented by following formulae



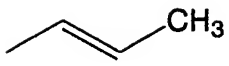




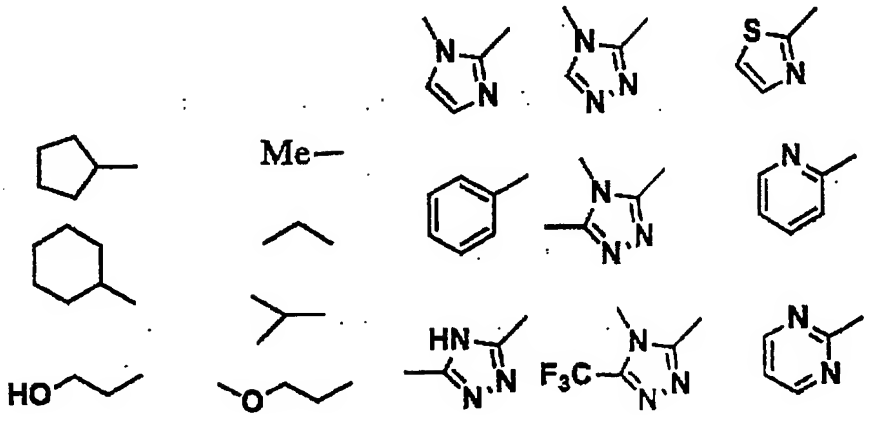
More preferred hydrocarbon group is methyl group, ethyl group, propyl group, isopropyl group or a group represented by following formulae



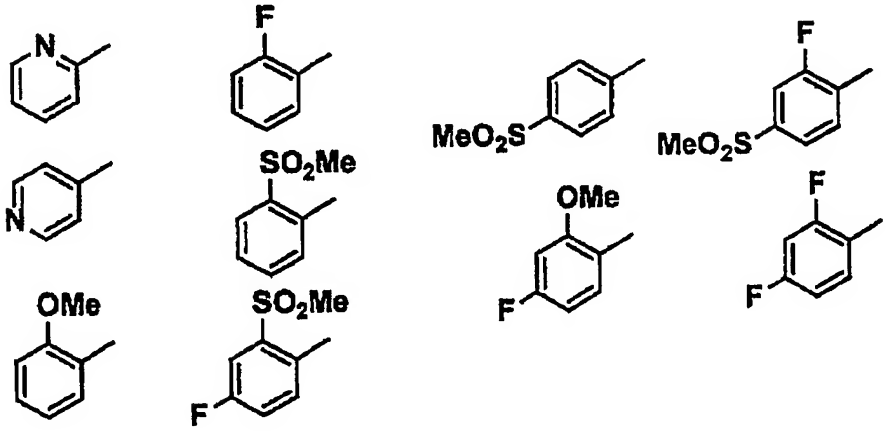
or



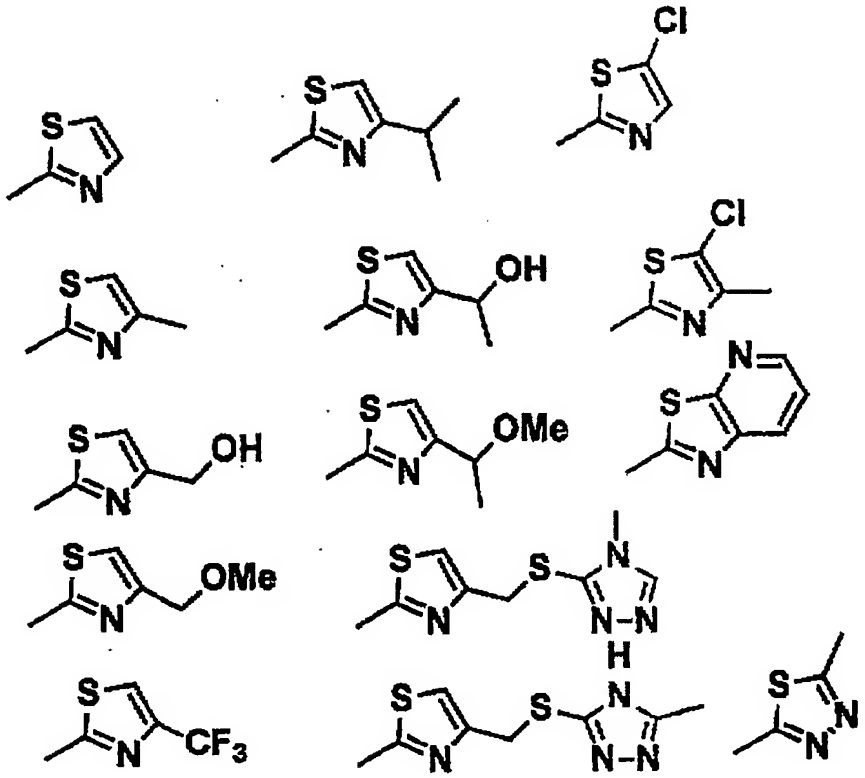
For example, as preferred A (in case of p = 0), the following groups are nominated.



As preferred B, for example, the following groups are nominated.



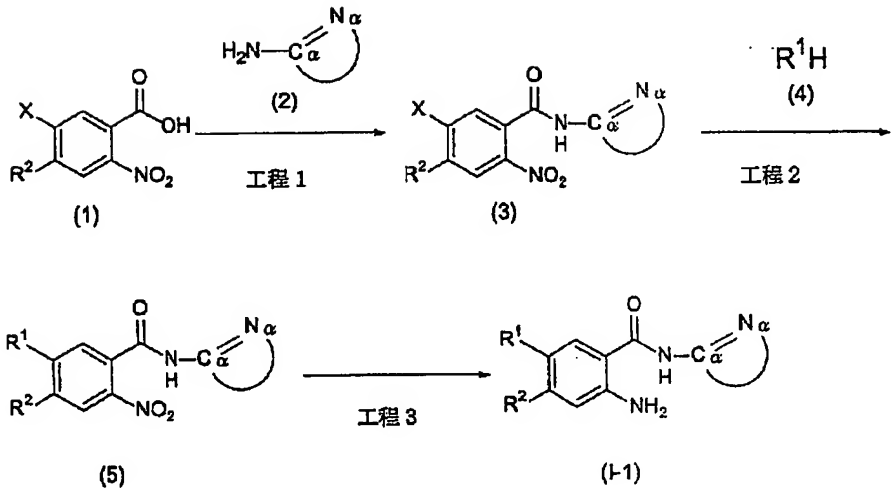
As heteroaryl group represented by formula (II), for example following heterocyclic groups are nominated.



Moreover, particularly preferred compounds are any of the compound represented by aforesaid formulae (IIIa)-(IIIc).

The compound of this invention (1) can be readily produced by using well known reaction means or according to well known method. Moreover, the compound of general formula (I) of this invention can be produced not only by synthesis in ordinary liquid phase, but also by synthesis using solid phase developed remarkably in recent years such as combinatorial synthesis method, parallel synthesis method or the like. Preferably, it can be produced for example using the following process.

Step 1 to Step 2 to Step 3



(wherein, each symbol is the same as in the aforesaid definition)

Step 1

This step is a process to produce compound (3) by reacting carboxylic acid compound (1) or reactive derivative thereof and amino compound containing optionally substituted monocyclic or bicyclic heteroaryl group represented by aforesaid formula (2) or salts thereof. In this reaction, ordinary amide formation reaction may be carried out by a method described in literature (for example Base and experiment of peptide synthesis, Shinya Izumiya et al., Maruzen, 1983, Comprehensive Organic Synthesis, vol 6, Pergamon Press Co. 1991 and the like) or in accordance with these, or by combining these and conventional method, in other word, it can be carried out by using condensing agent well-known for a person skilled in the art or by ester activation method, mixed acid anhydride method, acid chloride method, carbodiimide method and the like which can be used by a person skilled in the art. As such amide forming reagent, for example thionyl chloride, N,N-dicyclohexylcarbodiimide, 1-methyl-2-bromo pyridinium iodide, N,N'-carbonyldiimidazole, diphenyl phosphoryl chloride, diphenyl phosphoryl azide, N,N'-disuccinimidyl carbonate, N,N'-disuccinimidyl oxalato, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride, ethylchloroformate, chloro formic acid isobutyl ester or benzo triazol-1-yl-oxy-tris (dimethylamino) phosphonium hexafluoro phosphate and the like are proposed, and wherein, for example thionyl chloride, N,N-dicyclohexylcarbodiimide or benzo triazol-1-yl-oxy-tris (dimethylamino) phosphonium hexafluoro phosphate and the like are suitable. Moreover, in amide forming reaction, a base, a condensation assistant may be used with the aforesaid amide forming reagent.

As base used, for example tertiary aliphatic amine such as trimethylamine, triethylamine, N,N-diisopropyl ethylamine, N-methylmorpholine, N-methylpyrrolidine, N-methylpiperidine, N,N-dimethylaniline, 1,8-diazabicyclo[5.4.0] undec-7-ene (DBU), 1,5-azabicyclo[4.3.0] non-5-ene (DBN) or the like, for example aromatic amine such as pyridine, 4-dimethylaminopyridine, picoline, lutidine, quinoline, isoquinoline and the like are proposed, and wherein, for example tertiary aliphatic amine and the like is preferred, and in particular, for example triethylamine or N,N-diisopropyl ethylamine and the like is suitable.

As condensation assistant used, for example N-hydroxybenzotriazole hydrate, N-hydroxy succinimide, N-hydroxy-5-norbornene-2,3-dicarboximide or 3-hydroxy-3,4-dihydro-4-oxo-1,2,3-benzotriazole and the like are proposed, and among these, for example N-hydroxybenzotriazole and the like are suitable.

The amount of amino compound (2) used differs depending on the kind of compound and solvent used and other reaction conditions, however, usually, 0.02 to 50 equivalents, preferably 0.2 to 2 equivalents with respect to 1 equivalent of carboxylic acid compound (1) or reactive derivative thereof. Herein, as reactive derivative, for example active ester derivative, active amide derivative and the like which are used in the sphere of usual organic chemistry are nominated.

The amount of used amide forming reagent differs depending on the kind of compound and solvent used and other reaction conditions, however, usually 1-50 equivalents, preferably 1-5 equivalents with respect to 1 equivalent of carboxylic acid compound (1) or reactive derivative thereof.

The amount of used condensation assistant differs depending on the kind of compound and solvent used and other reaction conditions, however, usually it is 1-50 equivalents, preferably 1-5 equivalents with respect to 1 equivalent of carboxylic acid compound (1) or reactive derivative thereof.

The amount of used base differs depending on the kind of compound and solvent used and other reaction conditions, however, usually 1 to 50 equivalents, preferably 3 to 5 equivalents.

The reaction solvent used in this step, is for example insert organic solvent, and it is not restricted in

particular so long as it does not hinder the reaction. However, in an embodiment, for example methylene chloride, chloroform, 1,2-dichloroethane, trichloroethane, N,N-dimethylformamide, acetic acid ethylester, acetic acid methylester, acetonitrile, benzene, xylene, toluene, 1,4-dioxane, tetrahydrofuran, dimethoxyethane or a mixed solvent thereof are proposed, however, in particular for example methylene chloride, chloroform, 1,2-dichloroethane, acetonitrile, N,N-dimethylformamide or the like are suitable in term of securing a suitable reaction temperature.

The reaction temperature is -100°C to boiling point of solvent, preferably 0 to 30°C.

The reaction time is 0.5 to 96 hours, preferably 3 to 24 hours.

The base, amide formation reagent, condensation assistant used in this step 1 can be used as a single species or in combination of two or more.

When the compound (3) contains protecting group, said protecting group can be suitably eliminated. Elimination of aforesaid protecting group can be carried out by method described in literature (Protective Groups in Organic Synthesis, written by T.W. Green, the second edition, John Wiley & Sons Co, 1991, or the like) or method in accordance with this or by combining these and conventional method.

Compound (3) obtained in this way can be provided for the next step by isolating and purifying with well known separation and refinement means, for example concentration, vacuum concentration, crystallisation, solvent extraction, re-precipitation, chromatography and the like or without isolating and purifying.

Step 2

This step comprises a process to produce compound (5) by reacting amide compound (3) obtained in aforesaid step 1 and compound (4).

In this reaction, a base may be added to the reaction system in accordance with requirements. As used compound (4), preferably phenol derivative or thiol derivative is preferred. As said phenol derivative or thiol derivative, for example phenol, thiophenol, thio imidazole, thio triazole and the like are

nominated. The amount of compound (4) used differs depending on the kind of compound and solvent used or other reaction conditions, however, usually it is 2-50 equivalents, preferably 2-5 equivalents with respect to 1 equivalent of amino derivative (3). As used base, for example tertiary aliphatic amine such as trimethylamine, triethylamine, N,N-diisopropyl ethylamine, N-methylmorpholine, N-methylpyrrolidine, N-methylpiperidine, N,N-dimethylaniline, 1,8-diazabicyclo[5.4.0] undec-7-ene (DBU), 1,5-azabicyclo[4.3.0] non-5-ene (DBN) or the like, for example aromatic amine such as pyridine, 4-dimethylaminopyridine, picoline, lutidine, quinoline, isoquinoline and the like, alkali metal such as metallic potassium, metallic sodium, metallic lithium and the like, alkali metal hydride such as sodium hydride, potassium hydride and the like, alkali metal alkylate such as butyl lithium and the like, alkali metal alkoxide such as potassium-tert-butyrate, sodium ethylate or sodium methylate and the like, alkali metal hydroxide such as potassium hydroxide, sodium hydroxide and the like, alkali metal carbonate such as potassium carbonate and the like are nominated, among these for example tertiary aliphatic amine, alkali metal hydride or alkali metal carbonate are preferred, and in particular, for example triethylamine, N,N-diisopropyl ethylamine, sodium hydride or potassium carbonate are suitable.

The amount of aforesaid base used differs depending on the kind of compound and solvent used and other reaction conditions, however, it is usually 0 to 50 equivalents, preferably 2-10 equivalents with respect to 1 equivalent of amide compound (3). Said base can be used as a single species or two or more species in accordance with requirements.

As used insert organic solvent, there are no restrictions in particular so long as the reaction is not hindered. However, in an embodiment, for example methylene chloride, chloroform, 1,2-dichloroethane, trichloroethane, N,N-dimethylformamide, N,N-dimethyl acetamide, acetic acid ethylester, acetic acid methylester, acetonitrile, benzene, xylene, water, toluene, 1,4-dioxane, tetrahydrofuran, or a mixed solvent thereof are proposed.

Compound (5) obtained in this way can be and isolated and purified with well known separation and refinement means, for example concentration, vacuum concentration, crystallisation, solvent extraction, re-precipitation, chromatography and the like.

Step 3

This step is a process to produce compound (I) used in this invention by reduction of compound (5). As for reductive reaction used in this step, well-known processes to a person skilled in the art are used. As the reductive reaction used in this step, in an embodiment, for example (1) catalytic reduction method using hydrogen, formic acid, ammonium formate, hydrazine hydrate and palladium, platinum, nickel catalyst, (2) reduction method using hydrochloric acid, ammonium chloride and iron, (3) reduction method using methanol and tin chloride are nominated.

The amount of reducing agent used in the aforesaid reductive reaction differs depending on the kind of compound and solvent to be used and other reaction conditions, however, it is usually 1-50 equivalents, preferably 2-20 equivalents with respect to 1 equivalent of compound (5).

The reaction solvent used is not restricted in particular so long as the reaction is not hindered. However, for example halogenated hydrocarbons such as dichloromethane, chloroform and the like, ethers such as diethyl ether, tert-butyl methyl ether, tetrahydrofuran and the like, amides such as N,N-dimethylformamide, N,N-dimethylacetamide and the like, sulphoxides such as dimethylsulfoxide and the like, nitriles such as acetonitrile and the like, an alcohol such as methanol, ethanol, propanol and the like, aromatic hydrocarbons such as benzene, toluene, xylene and the like, water or mixed solvent thereof can be used.

Reaction temperature and the reaction time are not restricted in particular. However, the reaction is carried out for 1-20 hours approx. and preferably 1 to 5 hours approx. at a reaction temperature of -10 to 100°C approx. and preferably 0 to 50°C approx.

Compound (1) used in this invention obtained in this way can be provided for the next step by isolating and purifying with well known separation and refinement means, for example concentration, vacuum concentration, crystallisation, solvent extraction, re-precipitation, chromatography and the like or without isolating and purifying.

Compound of aforesaid each step may contain protecting group on each substituent. Aforesaid protecting group can be suitably eliminated in each step using well known method, method in accordance with that or method combined these and the conventional method. As for the embodiment of

elimination, suitable elimination reaction is possible depending on the kind of compound, reaction and other reaction conditions. However, it is considered the case in which each protecting group is eliminated individually and the case in which each protecting group is simultaneously eliminated and the like, and it can be suitably selected by a person skilled in the art. As aforesaid protecting group, for example protecting group of hydroxy group, protecting group of amino group, protecting group of carboxyl group, protecting group of aldehyde, protecting group of keto group and the like are nominated. Moreover, the order of elimination aforesaid protecting groups is not limited in particular.

As protecting group of hydroxy group, for example lower alkyl silyl group such as tert-butyldimethylsilyl group, tert-butyl diphenyl silyl group and the like, for example lower alkoxymethyl group such as methoxy methyl group, 2-methoxyethoxymethyl group and the like, for example aralkyl group such as benzyl group, p-methoxybenzyl group and the like, for example acyl group such as formyl group, acetyl group and the like are proposed, and among these, tert-butyldimethylsilyl group, acetyl group and the like are in particular preferred.

As protecting group of amino group, for example aralkyl group such as benzyl group, p-nitrobenzyl and the like, for example acyl group such as formyl group, acetyl group and the like, for example lower alkoxycarbonyl group such as ethoxycarbonyl group, tert-butoxycarbonyl group and the like, for example aralkyloxy carbonyl group such as benzyloxycarbonyl group, p-nitrobenzyl oxycarbonyl group and the like are proposed, and among these, nitrobenzyl group, tert-butoxy carbonyl group, benzyloxycarbonyl group and the like are particularly preferred.

As protecting group of carboxyl group, for example lower alkyl group such as methyl group, ethyl group, tert-butyl group and the like, for example aralkyl group such as benzyl group, p-methoxybenzyl group and the like are nominated, and among these, methyl group, ethyl group, tert-butyl group, benzyl group and the like are particularly preferred.

As protecting group of keto group, for example dimethyl ketal group, 1,3-dioxirane group, 1,3-dioxolane group, 1,3-dithiane group, 1,3-dithiorane group and the like are proposed, and among these, dimethyl ketal group, 1,3-dioxolane group and the like are more preferred.

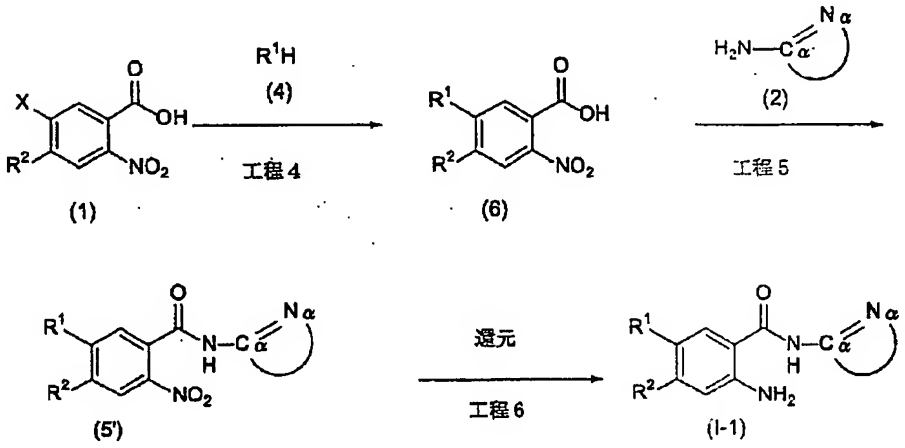
As protecting group of aldehyde group, for example, dimethylacetal group, 1,3-dioxirane group, 1,3-dioxolane group, 1,3-dithiane group, 1,3-dithiorane group and the like are proposed, and among these, dimethylacetal group, 1,3-dioxolane group and the like are more preferred.

In the production of compound used in this invention, there is a case that the protecting group is introduced to functional group in order to proceed reaction with good efficiency. The introduction of these protecting groups can be suitably selected by a person skilled in the art, and elimination of aforesaid protecting groups can be carried out by a method described in aforesaid Protective Groups In Organic Synthesis and the like, a method in accordance with that or by combining that and conventional method. Moreover, the order of elimination of protecting groups can be suitably selected by a person skilled in the art.

Compound (1) obtained in this way can be subjected to the next step after isolating and purifying with well known separation and refinement means, for example concentration, vacuum concentration, crystallisation, solvent extraction, re-precipitation, chromatography and the like or without isolating and purifying.

Moreover, the compound used in this invention (I) can be also produced by the following step.

Step 4 to Step 5 to Step 6



(wherein, each symbol has same aforesaid definition).

As far as the aforesaid Step 4, Step 5 and Step 6 are concerned, it can be carried out using the same amount of reagent, reaction solvent, reaction temperature and other reaction conditions as in aforesaid Step 1, Step 2 and Step 3.

When a protecting group is necessary for R2, it can be carried out by a person skilled in the art suitably selecting a process from a method described in aforesaid Protective Groups In Organic Synthesis and the like, a method in accordance with that or by combining that and conventional method.

Compounds (6) and (5') obtained in this way can be provided for the next step after isolating and purifying with well known separation and refinement means, for example concentration, vacuum concentration, crystallisation, re-precipitation, solvent extraction and the like or without isolating and purifying.

Compound (1) used in this invention can be isolated and purified by well known separation and refinement means, for example concentration, vacuum concentration, crystallisation, re-precipitation, solvent extraction and the like.

In aforesaid step 1 to 6, the elimination of protecting groups differ depending on the kind of aforesaid protecting group and stability of compound, however, it can be carried out by aforesaid method described in Protective Groups in Organic Synthesis, written by T.W. Green, the second edition, John Wiley & Sons Co, 1991, or the like or a method in accordance with this or by combining these and conventional method. For example, it can be carried out by solvolysis using acid or base, chemical reduction using hydrogenated metallic complex and the like or catalytic reductions using palladium carbon catalyst, Raney nickel and the like.

The benzamide compound put forward by this invention can exist as pharmacologically acceptable salt. Aforesaid salt can be produced in accordance with conventional methods. In an embodiment, when aforesaid compound (1) contains basic group derived from for example amino group, pyridyl group within the molecule, it can be converted to corresponding pharmacologically acceptable salt by treating aforesaid compound with an acid.

As aforesaid acid addition salt, for example the acid addition salt of halide acid salt such as hydrochloride, hydrofluoric acid salt, hydrobromic acid salt, hydroiodic acid salt or the like, inorganic acid salt such as nitrate, perchlorate, sulfate, phosphate, carbonate or the like, lower alkyl sulfonate such as methanesulfonate, trifluoromethanesulfonate, ethanesulfonic acid salt or the like, aryl sulfonate such as benzensuplhonate, p-toluenesulfonate or the like, organic salt such as fumarate, succinate, citrate, tartrate, oxalate, maleate or the like and organic acid of amino acid or the like such as glutamic acid salt, aspartate or the like are nominated. Moreover, when the compound of this invention is containing acidic group in aforesaid group, for example when containing carboxyl groups, it can be converted to corresponding pharmacologically acceptable salt by treating aforesaid compound with a base. As aforesaid base addition salt, for example salt of alkali metal salt such as sodium, potassium and the like, alkaline earth metal salt such as calcium, magnesium and the like, organic base such as ammonium salt, guanidine, triethylamine, dicyclohexylamine and the like are nominated. Furthermore, the compound of this invention may exist as free compound or arbitrary hydrate or solventate of salts thereof.

In accordance with this invention, as explained in detail in the description of Examples, crystal of complex of GK protein containing amino acid sequence shown in Sequence Number 5 and compounds of aforesaid formula (IIIa)-formula (IIIc) are obtained. In GK protein shown in Sequence Number 5, it has been elucidated that compound binding site is constituted from the amino acid residue of tyrosine 61 - serine 69, glutamic acid 96 - glutamine 98, isoleucine 159, methionine 210 - tyrosine 215, histidine 218 - glutamic acid 221, methionine 235, arginine 250, leucine 451 - lysine 459 by analysing these crystal three - dimensional structure coordinates.

Moreover, according to another embodiment of this invention, a process for the production of crystal containing a complex of protein and a compound that binds to the protein, wherein a protein production step to produce a protein containing amino acids sequence in which prescribed number of amino acid residues at N terminal side and/or C terminal side are deleted as described above from the protein containing amino acid sequence in accordance with Sequence Number 2 and a step to cause reaction of a compound which binds to the protein obtained in aforesaid protein production step with the protein obtained in said protein production step are included, is put forward.

As protein to be produced in the aforesaid protein production step, the number thereof is not restricted as long as it is within a range that steric hindrance between adjacent GK proteins in the crystal is eliminated. In an embodiment, for example, in the amino acids sequence shown in Sequence Number 2, the amino acids sequences in which amino acid residues at N terminal side are deleted in numbers of 1-50, preferably 3-30, more preferably 5-25, still more preferably 8-18, even more preferably 11-15 or the like are nominated. Moreover, the amino acid sequences in which amino acid residues at C terminal side of 1-8, preferably 1-7, more preferably 2-6 or the like are deleted, are nominated.

(The drug design process using three-dimensional structural coordinate).

Three-dimensional structure of GK protein of this invention obtained as above provides important information for drug creation system using CARDD (Computer Aided Rational Drug Design). It is an important step of the target drug creation and development to elucidate the active center and allosteric site of this GK protein and to search for a compound which is compatible to said site, interacts with the GK protein and thereby activates to inhibits the GK protein.

In other words, according to another embodiment of this invention, a drug design process of the kind to design structure of compound that binds to said protein based on stereostructure information of protein, characterised in that the stereostructure information of said protein comprises information to be obtained by analysing the crystal obtained as described above, is put forward. As such drug design process, there are techniques to make drug design using energy calculation, activity prediction value analogous to this or pharmacophore and a technique to visually design drug using computer graphics technique.

As process by technique using energy calculation, activity prediction value analogous to this or pharmacophore, (1) a drug design process including a binding site deduction step to deduce compound binding site of said protein based on stereostructural information obtained as above and a selection step to select a compound compatible to the compound binding site deduced in aforesaid binding site deduction step from the compound library, (2) a drug design process including a binding site deduction step to deduce compound binding site of said protein based on aforesaid stereostructural information and a compound structure assembly step to construct a structure of compound compatible to the compound binding site deduced in aforesaid binding site deduction step, or the like are exemplified.

As process to deduce compound binding site of aforesaid protein, for example, a process wherein the ligand bonded site in the co-crystal of compound is identified by confirming with visual observation on display of computer, and in addition to that, a process wherein the site to which ligand is likely to bind is identified with respect to the protein crystal structure solved under the condition that ligand is not bound, are nominated. In any processes, well-known method and commercial computer soft wear can be used. In former process, for example, it is possible to use software such as Insight II (Accelrys Inc.), SYBYL (Tripos Inc.), MOE (Chemical Computing Group) or the like. On the other hand, For example, in latter process, well known technique such as Cavity search: an algorithm for the isolation and display of cavity-like binding regions, (Journal of Computer-Aided Molecular Design. 4(4): 337-54, 1990) or the like can be used, and it can be carried out using software such as SiteID (Tripos Inc.) or the like.

Once the binding site of compound in protein was able to be deduced, a compound which can be compatible to the deduced binding site is selected. As process to select this candidate compound, structural information of the compound is acquired from existing compound library, and bindable candidate compound is selected by comparing the structural information of compound in the library and structural information of the binding site deduced as above.

In a further embodiment, 1 or more residues of amino acid residues of amino acid sequence shown in Sequence Number 5 (tyrosine 61 - serine 69, glutamic acid 96 - glutamine 98, isoleucine 159, methionine 210 - tyrosine 215, histidine 218 - glutamic acid 221, methionine 235, arginine 250, leucine 451 - lysine 459) or pharmacophore of hydrogen bonding or hydrophobic properties or the like formed from the functional group of ligand in the complex, and also the protein surface produced from the protein structure or a structure in which the orientation of a part of the side chain thereof is modified, are used as search condition, and the conformation and orientation of each compound is systematically searched from the compound library, and whether the conditions are satisfied or not is judged and it is selected.

As an alternative process, while systematically searching the conformation and orientation of each compound from the compound library, the candidate compound is virtually docked with respect to the structure of ligand binding site constructed from the amino acid residues (tyrosine 61 - serine 69, glutamic acid 96 - glutamine 98, isoleucine 159, methionine 210 - tyrosine 215, histidine 218 - glutamic acid 221, methionine 235, arginine 250, leucine 451 - lysine 459) or a structure in which the orientation

of a part of the side chain thereof is modified, the species that formed interaction of close proximity of 4 Å or less with 1 or more residues of amino acid residues of amino acid sequence (tyrosine 61 - serine 69, glutamic acid 96 - glutamine 98, isoleucine 159, methionine 210 - tyrosine 215, histidine 218 - glutamic acid 221, methionine 235, arginine 250, leucine 451 - lysine 459) is selected, or selection is carried out using energy evaluation function.

On the other hand, the candidate compound can also be selected by designing a bindable compound based on the structural information of the binding site deduced as above. In a further embodiment, each atomic species and functional groups are connected so that interactions are possible with respect to the structure of ligand binding site constructed from the amino acid residues (tyrosine 61 - serine 69, glutamic acid 96 - glutamine 98, isoleucine 159, methionine 210 - tyrosine 215, histidine 218 - glutamic acid 221, methionine 235, arginine 250, leucine 451 - lysine 459) or a structure in which the orientation of a part of the side chain thereof is modified, and thereby a chemical structure is constructed. As this process, a process wherein chemical groups such as methyl, ethyl and the like are arranged in the active site and a compatible compound is searched, and a process wherein atoms are bonded at active site using a computer program.

Moreover, with the process by energy evaluation using computer, for example a process to determine the bond energy of a compound and GK protein using molecular force field calculation is nominated. The calculation thereof is applied to each compound in database, and candidate compounds which can form stable binding are selected from the library compound. With some computer programs, such as Ludi of Insight II, when three-dimensional structural coordinates of interacting amino acid residues in the protein molecule are input, candidate of bindable compounds are automatically selected and output, and it can be suitably used.

Moreover, as far as the drug design on the basis of three-dimensional structure of molecule is concerned, many literature are known including development of pharmaceutical Vol. 7 "molecular design" (Hirokawa Shoten). In an embodiment, first, using flexible ligand binding simulation software such as for example FlexiDock, FlexX or the like, a library of low molecular (molecular weight 1000 or less) compounds (for example about 150000 species) can be screened with computer. For chemical compounds in this library, three-dimensional structure is built using a program such as CONCORD or the

like, and compounds compatible to the active site can be selected.

On the other hand, as a process of visual drug design, a drug design process characterised in including a binding site deduction step to deduce compound binding site of said protein based on aforesaid stereostructural information and a design step wherein the structure of the compound is visually designed so that aforesaid compound binding site deduced in aforesaid binding site deduction step and a compound compatible to said compound binding site can interact, is nominated. For example, structure assembly or structure modification is carried out with respect to the structure of ligand binding site constructed from the amino acid residues (tyrosine 61 - serine 69, glutamic acid 96 - glutamine 98, isoleucine 159, methionine 210 - tyrosine 215, histidine 218 - glutamic acid 221, methionine 235, arginine 250, leucine 451 - lysine 459) or a structure in which the orientation of a part of the side chain thereof is modified, so that it can interact with 1 or 2 or more residues among these residues.

In an embodiment, with visual process, first, crystal structure of the complex of GK protein and a compound bound to this is displayed on a computer screen according to the obtained structural coordinates. And, while considering the chemical interaction, the binding possibilities of the compounds in the library and GK protein are successively examined on computer. Wherein, the chemical interactions to be considered are electrostatic interaction, hydrophobic interaction, hydrogen bonding, van der Waals interaction or the like. In other words, the structure in three dimensional space of said compound is generally considered whether a structure favourable for the interaction is formed or not, so that among the functional groups thereof, the groups likely to be negatively charged such as carboxyl group, nitro group, halogen group or the like interact with amino acid residues having positive charge such as lysine, arginine, histidine of GK protein, the groups likely to be positively charged such as amino group, imino group, guanidyl group or the like interact with amino acid residues having negative positive charge such as glutamic acid, aspartic acid of GK protein, hydrophobic functional groups such as aliphatic group and aromatic group interact with hydrophobic amino acid residues such as alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine, the groups which participate in hydrogen bond such as hydroxy group, amide group or the like can for hydrogen bonding with the main chain or side chain part of the GK protein, furthermore steric hindrance is not caused by the binding of said compound and GK protein, moreover, furthermore, the void part is filled so that the void part is eliminated as much as possible so that the van der Waals interaction is increased, or the like. In this way,

the factors such as electrostatic interaction, hydrophobic interaction, van der Waals interaction, hydrogen bond or the like are comprehensively considered visually on the computer screen, and finally, whether the candidate compound can bind to the GK protein or not is determined.

As program for selecting compound candidate by visual observation in this way, simulation programs such as Insight II and MOE or the like are exemplified. In order to generate promising candidate compounds that interact with GK protein, the candidate compounds are contacted with GK protein, and activity of GK protein is measured. In practice, the promising candidate compound is mixed with GK protein, crystallised, and whether it is compatible or not is evaluated. Further, more desirable structure is formed by modifying the compatible complex using organic synthesis.

Moreover, the visual technique and the technique that considers energy may be suitably combined, and used. As such computer software, flexiDock (Tripos Inc.), FlexX (Tripos Inc.), SYBYL (Tripos Inc.), Insight II (Accelrys Inc.), MOE (Chemical Computing Group Inc.) or the like are nominated.

Moreover, in accordance with this invention, the compounds selected by aforesaid drug design process are synthesized, and these compound groups can be provided as compound array (compound library). Because a large quantity of candidate compounds can be assayed at one time using a technique such as high through-put screening or the like, the inhibitor or activator of glucokinase can be screened with good efficiency.

(Compounds obtained by process of this invention and therapeutic agent including these)

The compounds designed by aforesaid drug design process have has ability to bind to glucokinase, therefore they can be used as activators of glucokinase or glucokinase inhibitors. Moreover, the therapeutic agent or medicinal composition containing such compound can be effectively used as therapeutic agent of disease involving glucokinase activity (for example diabetes mellitus therapeutic agent).

Aforesaid medicinal composition contains a compound that binds to glucokinase of this invention as effective ingredient in pharmacologically effective dose thereof together with suitable pharmacologically permitted support or diluent. As the pharmacologically acceptable support which can be used in aforesaid

medicinal composition (drug formulation), diluent such as filler, extender, binding agent, humectant, disintegrating agent, surface active agent, lubricant or the like which are conventionally used corresponding to the form of the formulation or excipient or the like are exemplified. These carriers can be suitably selected and used corresponding to administration unit form of the obtained formulation.

As administration unit form of medicinal composition of this invention, various forms can be selected according to therapeutic purpose, and, as representative examples thereof, solid administrative form such as tablet, pill, powder, powder agent, granule, encapsulated formulation or the like and liquid agent administrative form such as solution, suspending agent, emulsion, syrup, elixir or the like are included. Further these are classified into orally administered agent, aoral drug, transnasal agent, vaginal agent, suppository, sublingual agent, ointment or the like according to administration route, and it can be formulated, molded and prepared each according to conventional process. For example, when it is formed to a tablet form, excipient such as lactose, lactose, refined sugar, sodium chloride, glucose, urea, starch, calcium carbonate, kaolin, crystalline cellulose, silicic acid, potassium phosphate or the like, binding agent such as water, ethanol, propanol, simple syrup, glucose syrup, starch solution, gelatin solution, carboxymethylcellulose, hydroxypropylcellulose, methylcellulose, polyvinylpyrrolidone or the like, disintegrating agent such as carboxymethylcellulose sodium, carboxymethylcellulose calcium, low degree of substitution hydroxypropylcellulose, dried starch, sodium alginate, agar powder, laminaran powder, sodium bicarbonate, calcium carbonate or the like, surface active agent such as polyoxyethylene sorbitan fatty acid ester species, lauryl sodium sulfate, stearic acid monoglyceride or the like, disintegration inhibitor such as refined sugar, stearin, cacao butter, hydrogenation oil or the like, absorption accelerating agent such as quaternary ammonium base, sodium lauryl sulfate or the like, humectant such as glycerol, starch or the like, adsorbent such as starch, lactose, kaolin, bentonite, colloidal silica or the like, lubricant or the like such as purified talc, stearate, boric acid powder, polyethyleneglycol or the like can be used. Further, the tablet can be formed into a tablet coated with ordinary agent coating in accordance with requirements, for example sugar coated tablet, gelatin encapsulation tablet, enteric coated tablet, film coatings tablet and moreover can be made into double layer tablet or multilayer tablet.

When a form of pill is formed, as formulation carrier, for example, excipient such as glucose, lactose, starch, cacao butter, hardened vegetable oil, kaolin, talc or the like, binding agent such as powdered gum

arabic, tragacanth powder, gelatin, ethanol or the like, disintegrating agent or the like such as laminaran, agar or the like can be used.

For encapsulated formulation, effective ingredient of this invention is mixed with the various formulation carrier exemplified as above according to normal method, and it is prepared by being packed into hard gelatin capsule, soft capsule or the like.

The liquid administration form for oral administration includes pharmacologically permitted solution, emulsion, suspension, syrup, elixir or the like containing generally used inert diluent, for example water, and furthermore, auxiliary such as wetting agent, emulsion, suspending agent or the like can be contained, and these are prepared according to normal method.

For the preparation of liquid administrative form for aoral administration, for example, sterile aqueous or non-aqueous solution, emulsion, suspension or the like, as diluent, for example water, ethanol, propylene glycol, polyethyleneglycol, ethoxylation isostearyl alcohol, polyoxyisosteary alcohol, polyoxyethylene sorbitan fatty acid ester and vegetable oil or the like such as olive oil or the like can be used, and moreover, injectable organic ester species, for example, ethyl oleate or the like can be formulated. Further, ordinary solubilser, buffer agent, wetting agent, emulsifier, suspending agent, preservative, dispersant or the like can be added to these. Sterilisation can be carried out for example by filtration operation through bacteria retaining filter, formulation of fungicide, irradiation treatment and heat treatment or the like. Moreover, these can be prepared as sterile solid composition which can be dissolved in sterile water or suitable sterilisable vehicle immediately before the use.

When forming into a form of suppository or vaginal administration, as formulation carrier, for example polyethyleneglycol, cacao butter, higher alcohol, higher alcohol ester, gelatin and semi-synthetic glyceride or the like can be used.

When forming into a form ointment such as paste, cream, gel or the like, as diluent, for example white petrolatum, paraffin, glycerol, cellulose derivative, propylene glycol, polyethyleneglycol, silicon, bentonite and vegetable oil or the like such as olive oil or the like can be used.

A composition for transnasal or sublingual administration can be prepared according to conventional method using standard excipient.

Moreover, in agent of this invention, colorant, preservative, flavor, flavor agent, sweetener or the like or other pharmaceutical or the like can be contained in accordance with requirements.

The amount of the effective ingredient to be contained in the aforesaid drug formulation and dose thereof are not restricted in particular, and it is suitably selected from a wide range corresponding to the desired therapy effect, administration method, therapy period, age, sex of patient, other conditions or the like. In general, the dose is about 0.01 mg - 1000 mg, preferably about 1 mg - 100 mg per 60 kg in weight per day usually, and it can be administered once or divided into several times per day.

Sequence number of sequence table of this specification shows following sequence.

(Sequence number: 1).
Base sequence of DNA encoding human derived liver type glucokinase is shown.

(Sequence number: 2).
Amino acid sequence of human derived liver type glucokinase is shown.

(Sequence number: 3).
Amino acid sequence of human derived beta cell glucokinase is shown.

(Sequence number: 4).
Base sequence of DNA encoding the protein in which 11 amino acid residues at N terminal side of human derived liver type glucokinase are deleted, is shown.

(Sequence number: 5).
Amino acid sequence of the protein in which 11 amino acid residues at N terminal side of human derived liver type glucokinase are deleted, is shown.

(Sequence number: 6).

Base sequence of primer-1 used in PCR reaction in the following Example 1 is shown.

(Sequence number: 7).

Base sequence of primer-2 used in PCR reaction in the following Example 1 is shown.

(Sequence number: 8).

Amino acid sequence of the protein in which 15 amino acid residues at N terminal side of human derived liver type glucokinase are deleted, is shown.

(Sequence number: 9).

Base sequence of the primer used in PCR reaction in the following Example 6 is shown.

(Sequence number: 10).

Base sequence of the primer used in PCR reaction in the following Example 6 is shown.

Examples

Hereinafter, this invention will be described in concrete terms using Examples.

A process for purification of mutant type enzyme

In human glucokinase, there are liver type and pancreas type depending on the difference of promoter, and 15 residues at N terminal are different. In order to carry out crystallisation for the purpose of three-dimensional structure analysis, a mutant type enzyme which lacked a part or a whole of this region was made by the following process.

PCR reaction was carried out using cDNA of human liver type glucokinase cloned on pCR2.1 (made by INTROGEN Co.) and two kinds of primer sets, comprising

a combination of 5'-gtcacaaggagccagaagcttatggccttgactctggtag-3' (sequence number 6) and 5'-gaagccccacgacattgttcccttctgc-3 (sequence number 7), and

a combination of 5'-ccaggcccagacagccaagcttatggtagagcagatcc-3' (sequence number 9) and 5'-gaagccccacgacattgttcccttctgc 3' (sequence number 10).

The Hind III, Cla I fragment of the obtained PCR product was substituted with Hind III-Cla I region of liver type GK cloned at Hind III, Eco RI site of pFLAG/CTC vector (Eastman Kodak), and thereby cDNAs encoding mutant type GK (Δ1-11) which lacked 1-11 residues of liver type GK and mutant type GK (Δ1-15) which lacked 1-15 residues were obtained. The sequence of the obtained DNA was confirmed, and thereafter, these vectors were made as expression vectors, and Escherichia coli DH alpha strain (made by Takara Shuzo company) was transformed.

Transformant was cultured in LB medium at 37°C till the absorption at 600 nm became 0.8, and thereafter, isopropyl-1-thio-beta-D-galactoside (made by Wako Pure Chemicals Co.) was added so as to become the final concentration of 0.4 mM, and the protein production was induced at 25°C for 16 hours.

Cultured Escherichia coli was collected using centrifuge, and it was suspended in a buffer containing the following components (50 mM potassium phosphate (Potassium Phosphate) pH 7.5, 50 mM NaCl, 2 mM DTT, 0.5 mM Pefabloc SC (made by Kanto Chemicals Company), a proteinase inhibitor mixture (made by Roche Co.)) .

Collected Escherichia coli was pulverised by ultrasonic pulverisation method, and soluble fraction was dialysed against aforesaid buffer, and thereafter, it was purified using HiTrapQ column (made by Amersham Corp.). The GK fraction eluted from HiTrapQ column by potassium chloride gradient was diluted to a salt concentration of 50 mM by dilution.

The diluted GK fraction was purified by Glucosamin Sepharose column produced by a process demonstrated in the paper (Preparative Biochemistry, 20(2), 163-178 (1990)). The GK fraction was adsorbed onto Glucosamin Sepharose column, and impurity was eliminated with 100 mM sodium chloride, and thereafter, it was eluted by glucose of 1 M.

The eluted GK fraction was refined by MonoQ10/10 column. The GK fraction eluted from the MonoQ10/10 column (made by Amersham Corp.) by sodium chloride gradient was purified by Superdex 200 column (made by Amersham Corp.) using 50 mM Tris-Cl pH 7.2, 50 mM NaCl buffer as mobile layer.

Crystallisation process

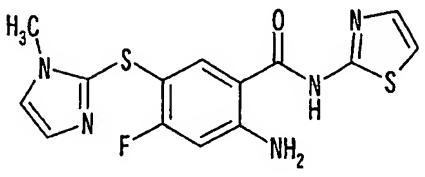
Crystal of mutant type GK [Δ1-11] / glucose / compound complex

The crystal of mutant type GK (Δ1-11) / glucose / compound complex was obtained using a technique of the following vapor diffusion. Moreover, mutant type GK (Δ1-11) denotes a glucokinase containing amino acid sequence represented by Sequence Number 5.

In other words, mutant type GK purified to a high purity was concentrated, and finally a solution of mutant type GK of about 10 mg/ml (25 mM Tris-Cl, 50 mM NaCl, 5 mM TCEP) was formed. Thereto were added glucose of final concentration 20 mM and following compound 1 (compound of formula IIIa) which activates GK of final concentration 0.3 mM, and this was used for crystallisation. To protein solution 1-5 μl was added as crystallisation solution, an equal quantity of 28-30 % PEG 1500 and 0.1 M Hepes-NaOH (pH 6.6), and this solution formed by admixing was placed in a closed container containing 0.5-1 ml of crystallisation solution as that both solutions did not form contact, and the container was left to stand at 20°C. After standing for about 3 days - 1 months, crystals with maximum size of about 0.4 mm x 0.4 mm x 0.7 mm was obtained in the sample solution (Example 1).

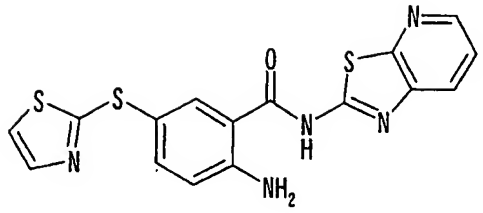
Furthermore, the crystals obtained by aforesaid method were immersed for about 3-7 days in 28-80 % PEG 1500, 0.1 M HePes-NaOH (pH 6.6) solution so that the following compound 2 (compound represented by formula IIIb) was contained in a concentration of 0.3 mM, and thereby a complex crystals of the following compound 2 and aforesaid mutant type GK were obtained.

Compound 1



(IIIa)

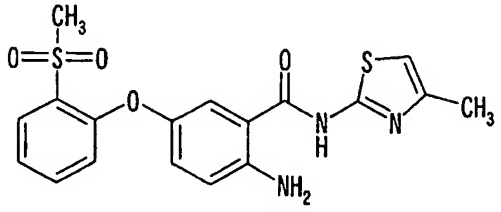
Compound 2



(IIIb)

Moreover, crystallisation was carried out in the same way as in Example 1 except that compound 3 (compound represented by formula IIIc) was used in stead of aforesaid compound 1, and as a result, crystals were respectively obtained in the same way as in Example 1 (Example 3).

Compound 3



(IIIc)

The obtained crystals were immersed into a crystallisation solution added with 10 % glycerol, thereafter it was rapidly frozen in liquid nitrogen. The X-ray diffraction data of the frozen crystal was collected in 100 K nitrogen gas stream by oscillation method at BL6B of synchrotron institution KEK-PF. From the obtained diffraction pattern, diffraction intensity was numerised using DENZO/SCALEPACK (made by HKL Co.), and crystal structure factor was determined. At this step, the crystal was found to be hexagonal system and had a space group of $P6_522$ or $P6_122$, and crystalline unit lattice was a = b = 79.9 angstrom, c = 322.2 angstrom, alpha = beta = 90°, gamma = 120°.

Using the obtained structure factor and three-dimensional structural coordinates of Human hexokinase type 1, the structure was analysed by molecular replacement method. Data with the resolution of 8 angstrom to 4 angstrom was used for the calculation, and it was performed by Amore program of CCP4 (Council for the Central laboratory of the Research Councils). The R factor of structure obtained by calculation was 53.7 %, and it was found that the space group of the crystal was $P6_522$ and a single molecule of mutant type GK was contained in asymmetrical unit. Electron density map was obtained

from this structure and structure factor, and the structure of mutant type glucokinase was determined using a program O (made by Dat-ONO Company).

Thereafter, refinement of the position of amino acid was carried out using CNX (Accelrys Inc.) and identification of amino acid residue was carried out using program O. This operation was repeated, and the structural coordinate of 448 amino acid residues from threonine 14 of the mutant type glucokinase to cysteine 461, 1 molecule of glucose molecule, 1 molecule of compound A, 1 sodium ion and 149 water molecules were identified, and the structural coordinates were determined. The R factor which is used as index of accuracy of finally determined structure was R = 23.2 % with respect to the data of resolution from 30 angstrom to 2.3 angstrom, and the R factor (Rfree) with respect to the data which was not used for the calculation in the refinement step of the structure was 27.4 %. There was no amino acid residue having the unacceptable structure by confirmation with Ramachandran plot.

The structure of the determined mutant type glucokinase was similar to the structure of the hexokinase which was isozyme, but the structure of the binding site of compound 1 (compound of formula IIIa) which activates glucokinase was different. This structural difference could not be expected with the ability of current computational chemistry and it became clear for the fist time by this structural analysis that this site was the binding site of activator and its detailed stereostructure. Figure 1a is ribbon diagram showing three-dimensional structure of the glucokinase elucidated here. As shown in Figure 1a, the newly found activator binding site was located between large domain and small domain, and it was about 20 angstrom away from the active center wherein glucokinase bonded with the substrate, glucose. The amino acid residue of glucokinase constituting the activator binding site was as follows. Tyrosine 61 - serine 69, glutamic acid 96 - glutamine 98, isoleucine 159, methionine 210 - tyrosine 215, histidine 218 - glutamic acid 221, methionine 235, arginine 250, leucine 451 - lysine 459.

Moreover, the binding scheme of compound 1 (compound of formula IIIa) with respect to this binding site is shown in Figure 2 and the structure of binding site of glucokinase is shown in Figure 3. The thiazole ring formed van der Waals contact with each amino acid side chain molecule of valine 62, valine 452, valine 455, and moreover the nitrogen atom on thiazole ring was hydrogen bonded with nitrogen atom of main chain of arginine 63. The nitrogen atom of amide on compound 1 was hydrogen bonded with oxygen atom of main chain of arginine 63. Benzene ring part of compound 1 was formed van der

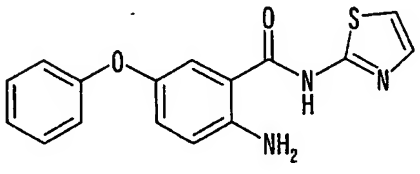
Waals contact with isoleucine 211, and the fluorine atom substituted to benzene ring formed van der Waals contacted with side chain of tyrosine 214. Aniline structure of compound 1 formed hydrogen bond with oxygen atom of side chain of tyrosine 215. Imidazole ring part bonded to the benzene ring via sulfur formed van der Waals contacted with amino acid side chain part of methionine 210, methionine 235, tyrosine 214. The serine 64-serine 69 part connecting the small domain and the large domain had a structure exposed to the solution, and compound 1 was bonded to the lower part of the arc-form structure formed by this part (Figure 3).

Example 4

Example of drug design

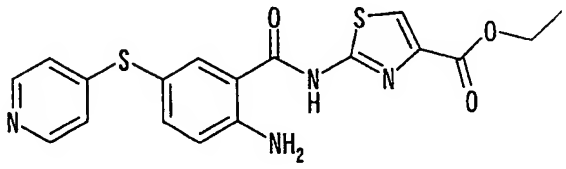
Using software UNITY (made by Tripos Company), pharmacophore of hydrogen bond acceptor and the hydrogen bond donor respectively generated from the main chain NH, CO of Arg 63, hydrophobic pharmacophore formed in the space corresponding to the phenyl group of aniline part of the ligand which formed the complex, and the protein surface formed on the basis of structure of the protein were used as search conditions, and Library compounds were screened, and the following compound 4 and compound 5 were obtained, and assay was carried out. As a result, activity of 780 % and 560 % was respectively observed. Wherein, the activity of 780 % denotes that the activity was enhanced upto 780 % by these compound when the activity of glucokinase of the control was 100 % (using glucose 2.5 M and ligand 10 μM).

Compound 4



Activity: 780 %

Compound 5



Activity: 560 %

Example 5

Crystal of mutant type GK (Δ1-15)

The crystal of simple substance of mutant type GK (Δ1-15) (glucokinase containing amino acid sequence represented by Sequence Number 8) was obtained using the following vapor diffusion technique.

In other words, mutant type GK purified to a high purity was concentrated, and finally a solution of mutant type GK of about 10 mg/ml (25 mM Tris-Cl pH 7.2, 50 mM NaCl, 5 mM TCEP) was formed. To protein solution 1-5 μl was added an equal quantity of crystallisation solution (1.5-1.6 M ammonium sulfate, 50 mM NaCl, 0.1 M Bicine NaOH (pH 8.7)), and this solution formed by admixing was placed in a closed container containing 0.5-1 ml of crystallisation solution as that both solutions did not form contact, and the container was left to stand at 20°C. After standing for about 3 days - 1 months, crystals with maximum size of about 0.07 mm x 0.07 mm x 0.5 mm was obtained in the sample solution.

The obtained crystals were immersed into the crystallisation solution added with 20 % glycerol, and continuing it was frozen rapidly in liquid nitrogen. The X-ray diffraction data of the frozen crystal were collected by oscillation method in 100K nitrogen gas stream at BL32B2 of synchrotron institution Spring-8. From the obtained diffraction image, the diffraction intensity was numerised using Mosflm, and crystal structure factor was determined. At this step, it became clear that the crystal was hexagonal system and had space group of $P6_522$ or $P6_122$, and crystal unit lattice was a = b = 103.2 angstrom, c = 281.0 angstrom, alpha = beta = 90°, gamma = 120°.

Next, molecular replacement method was carried out using the obtained structure factor and structure was analyzed. As model of stereostructure, three-dimensional structural coordinates of each domain of glucokinase determined from the mutant type GK (Δ1-11) / glucose / compound complex crystal was separately used. The calculation was performed by Amore program of CCP4 (Council for the Central laboratory of the Research Councils) using data of resolution of 8-4 angstrom. It was found that the space group of the crystal was $P6_522$, and a single molecule of mutant type GK (Δ1-15) was contained in the asymmetrical unit. Electron density map was obtained from this structure and structure factor, and the structure of mutant type GK (Δ1-15) simple substance was determined using program O (made by Dat-ONO Company).

Next, refinement of position of amino acid was carried out using CNX (made by Molecular Simulation Company) and identification of amino acid residue was carried out using program O. This operation was carried out repeatedly, and the structure coordinate of 424 amino acid residues from asparagine 180 to cysteine 461 and from methionine 15 to histidine 156 of mutant type glucokinase, and 2 molecules of sulfate ions, 1 sodium ion and 7 water molecules were identified, and the structural coordinates were determined. The R factor which is used as index of accuracy of finally determined structure was R = 23.8 % with respect to data of resolution of 50-3.4 angstrom, and the R factor (Rfree) with respect to the data which was not used for the calculation in the refinement step of structure was 30.6 %. There was no amino acid residue having the unacceptable structure by confirmation with Ramachandran plot.

The ribbon diagram showing the structure of glucokinase (Δ1-11) / glucose / compound 1 and the ribbon diagram showing the structure of glucokinase (Δ1-15) simple substance are respectively shown in Figure 1a and Figure 1b. Moreover, the figure on the right is a rotated figure of the figure on the left. In the structure of determined mutant type GK (Δ1-15) simple substance, the structures of main parts of the large domain and the small domain were similar to the respective structures of glucokinase determined by mutant type GK (Δ1-11) / glucose / compound complex crystal, but relative position of two domains was greatly different. In mutant type GK (Δ1-15) simple substance structure, the main part of the small domain was rotated by about 99 degrees from position of small domain in mutant type GK (Δ1-11) / glucose / compound complex structure. Moreover, alpha 13 helix located at C terminal region of glucokinase which constituted the small domain in the mutant type GK (Δ1-11) / glucose / compound complex structure no longer constituted the small domain in the mutant type GK (Δ1-15) simple substance structure, and it was located at between two domains. Moreover, because both the activator binding site and binding site of substrate, glucose were present between two domains in the mutant type GK (Δ1-11) / glucose / compound complex structure, the structures of their sites were greatly changed in the newly determined structure. The amino acid residues that play an important role in enzyme activity did not form active site in the mutant type GK (Δ1-15) simple substance structure, and the structure of mutant type GK (Δ1-15) simple substance analysed here was a structure of inactive state of glucokinase. Moreover, the activator binding site had completely disappeared in the structure of mutant type GK (Δ1-15) simple substance. The structural change of glucokinase (rotation of domains about 99°) observed by the mutant type GK (Δ1-11) / glucose / compound complex structure and the mutant type GK (Δ1-15)

simple substance structure was far greater compared with the previously known structural change of hexokinase (rotation of domains about 12°), and it could not be expected with the ability of current computational chemistry and it became clear from this structure analysis for the first time.

Moreover, in order to hinder the structural change to the inactive form mutant type GK (Δ1-15) simple substance structure, by designing a compound that binds to the compound binding site indicated by the mutant type GK (Δ1-11) / glucose / compound complex structure, it became clear that activator of glucokinase could be designed.

Possible Applications in Industry

As described above, in accordance with this invention, crystal of the glucokinase protein which was difficult to crystallise in the prior art was obtained. The three-dimensional structural coordinates obtained by analysing this crystal structure can be suitably used in order to design compounds that bind to glucokinase. Moreover, because the compounds designed in this way bind to glucokinase, they can be used as therapeutic agent of disease involving the glucokinase activity (for example diabetes mellitus therapeutic agent) as glucokinase activator or inhibitor.

Patent Claims

1. A glucokinase protein characterised in being used for crystallisation.

2. A protein in accordance with Claim 1 comprising amino acid sequence in accordance with Sequence Number 5.

3. A crystal of protein comprising amino acid sequence in accordance with Sequence Number 5 or amino acid sequence substantially the same amino acid sequence thereof.

4. A crystal in accordance with Claim 3, wherein the said protein is glucokinase protein.

5. A crystal in accordance with Claim 3 comprising crystals of protein containing amino acid sequence in accordance with Sequence Number 5.

6. A crystal in accordance with Claim 3, wherein the lattice constant satisfies the following equations (1)-(4)

a = b = 79.9 +/- 4 Å (1)

c = 322.2 +/- 15 Å (2)

alpha = beta = 90° (3)

gamma = 120° (4)

7. A crystal in accordance with Claim 6, wherein the space group is $P6_522$.

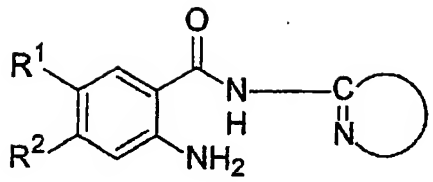
8. A crystal of protein specified by three-dimensional structure coordinates data in accordance with Table 1.

9. A crystal wherein in three-dimensional structure coordinates data changed in at least one data of three-dimensional structure coordinates data in accordance with Table 1, the mean square error between atoms of main chain of amino acid represented by three-dimensional structure coordinates data in accordance with Table 1 (C alpha atom) and C alpha atoms represented by the said changed three-dimensional structure coordinates data corresponding to aforesaid C alpha atoms is 0.6 Å or less.

10. A crystal in accordance with any of Claims 3-9, wherein the compound binding site is constructed by at least one of amino acid residues of tyrosine 61 - serine 69, glutamic acid 96 - glutamine 98, isoleucine 159, methionine 210 - tyrosine 215, histidine 218 - glutamic acid 221, methionine 235, arginine 250, leucine 451 - lysine 459 in amino acid sequence shown in sequence Number 5.

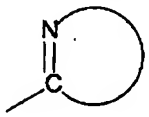
11. A crystal including a complex of the protein comprising amino acid sequence in accordance with Sequence Number 5 or amino acid sequence substantially the same amino acid sequence thereof and a compound which can bind to the said protein.

12. A crystal in accordance with Claim 11, wherein aforesaid compound is represented by formula (1).



(I)

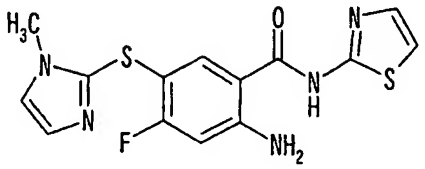
[wherein, R1 shows halogen atom, -S-(O)p-A, -S-(O)q-B or -O-B (wherein, p and q are the same or different and denote an integer of 0-2, A denotes C1-C6 alkyl group of optionally substituted straight chain, B denotes optionally substituted five-membered or six-membered ring aryl group or heteroaryl group, R2 denotes a hydrogen atom or halogen atom, and



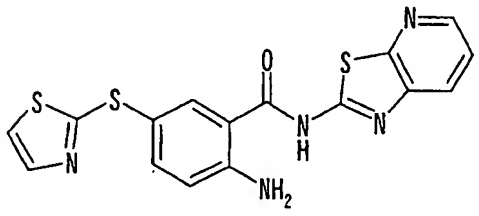
(II)

denotes an optionally substituted monocyclic or bicyclic heteroaryl group having a nitrogen atom adjacent to the carbon atom bonded to amide group].

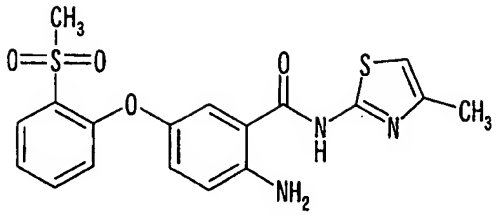
13. A crystal in accordance with Claim 12, wherein aforesaid compound is any of the compound represented by formula (IIIa)-(IIIc).



(IIIa)



(IIIb)



(IIIc)

14. A protein in accordance with Claim 1 comprising amino acid sequence in accordance with Sequence Number 8.

15. A crystal of protein comprising amino acid sequence in accordance with Sequence Number 8 or amino acid sequence substantially the same amino acid sequence thereof.

16. A crystal in accordance with Claim 15, wherein the said protein is glucokinase protein.

17. A crystal in accordance with Claim 15 comprising crystals of protein containing amino acid sequence in accordance with Sequence Number 8.

18. A crystal in accordance with Claim 15, wherein the lattice constant satisfies the following equations

a = b = 103.2 +/- 5 Å (5)

c = 281.0 +/- 7 Å (6)

alpha = beta = 90° (7)

gamma = 120° (8)

19. A crystal in accordance with Claim 18, wherein the space group is $P6_522$.

20. A crystal of protein specified by three-dimensional structure coordinates data in accordance with Table 2.

21. A crystal wherein in three-dimensional structure coordinates data changed at least one data of three-dimensional structure coordinates data in accordance with Table 2, the mean square error between atoms of main chain of amino acid represented by three-dimensional structure coordinates data in accordance

with Table 2 (C alpha atom) and C alpha atoms represented by the said changed three-dimensional structure coordinates data corresponding to aforesaid C alpha atoms is 0.6 Å or less.

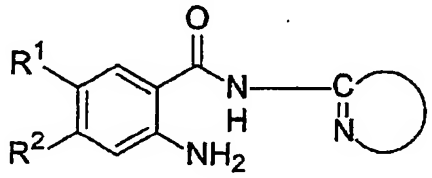
22. A process for the production of crystal containing a complex of protein and a compound that binds to the protein thereof, including

a protein production step wherein a protein containing the amino acid sequence having deletion of 1-50 amino acid residues from either or both of N terminal and C terminal of the protein containing amino acid sequence in accordance with Sequence Number 2 is produced, and

a protein reaction step wherein a compound that binds to the protein obtained in the said protein production step and the protein obtained in the said protein production step are reacted.

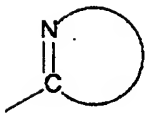
23. A process to produce crystal of the kind wherein a crystal of a protein is produced, characterised in that a protein including amino acid sequence in accordance with Sequence Number 5 or amino acid sequence substantially the same amino acid sequence thereof and having glucokinase activity and a compound which can bind to the said protein are used.

24. A process for the production of crystalline protein in accordance with Claim 23, wherein the compound which can bind to said protein is a compound represented by formula (1).



(I)

[wherein, R1 shows halogen atom, -S-(O)p-A, -S-(O)q-B or -O-B (wherein, p and q are the same or different and denote an integer of 0-2, A denotes C1-C6 alkyl group of optionally substituted straight chain, B denotes optionally substituted five-membered or six-membered ring aryl group or heteroaryl group, R2 denotes a hydrogen atom or halogen atom, and



(II)

denotes an optionally substituted monocyclic or bicyclic heteroaryl group containing nitrogen atom adjacent to the carbon atom bonded to amide group].

25. A process for the production of crystal in accordance with Claim 23 or 24 using co-crystallisation or soaking method

26. A drug design method of the kind wherein based on stereostructural information of a protein, the structure of compound that binds to said protein is designed, characterised in that the stereostructure information of said protein is the information obtained by analysing crystal in accordance with any of Claims 3-13 or 15-21.

27. A drug design method in accordance with Claim 26 characterised in that
a binding site deduction step wherein the compound binding site of said protein is deduced based on aforesaid stereostructure information, and
a selection step wherein a compound compatible to the compound binding site deduced in aforesaid binding site deduction step is selected from compound library,
are included.

28. A drug design method in accordance with Claim 26 characterised in that
a binding site deduction step wherein the compound binding site of said protein is deduced based on aforesaid stereostructure information, and
a compound structure assembly step wherein the structure of compound compatible to compound binding site deduced in aforesaid binding site deduction step is constructed,
are included.

29. A drug design method in accordance with Claim 26 characterised in that

a binding site deduction step wherein the compound binding site of said protein is deduced based on aforesaid stereostructure information, and

a design step wherein the structure of compound is designed by visual observation so that the compound binding site deduced in aforesaid binding site deduction step and a compound compatible to said compound binding site interact,

are included.

30. A drug design method in accordance with any of Claims 26-29, wherein aforesaid compound binding site is constituted by at least one of amino acid residue of tyrosine 61 - serine 69, glutamic acid 96 - glutamine 98, isoleucine 159, methionine 210 - tyrosine 215, histidine 218 - glutamic acid 221, methionine 235, arginine 250, leucine 451 - lysine 459 in amino acid sequence shown in sequence Number 5.

31. A drug design method in accordance with any of Claims 26-30 further including a step to measure physiological activity of the candidate compound predicted to be compatible to aforesaid compound binding site.

32. A drug design method in accordance with any of Claims 26-30 further including a binding determination step wherein the candidate compound predicted to be compatible to aforesaid compound binding site and a protein including amino acid sequence in accordance with and Sequence Number 5 or amino acid sequence which is substantially the same amino acid sequence thereof are contacted, and whether the candidate compound binds to the said protein or not is assessed.

33. A process for the production of compound array including the compound group selected by drug design method in accordance with any of Claims 26-30 is combined as compound array.

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